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REVIEW ARTICLE

Traffic Signal Analysis using Reinforcement Learning – A Review

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ABSTRACT

Traffic congestion has become a troublesome and difficult problem. The junctions where traffic bottlenecks are known to exist despite being historically signalized are of special importance. As Machine Learning (ML) algorithms allow the control system to automatically learn the optimal solutions, it is a promising approach for traffic congestion problems. Reinforcement Learning (RL) is a ML technique that has been used widely by various researchers to monitor and alleviate traffic congestion. RL allows autonomous decision-makers to watch, learn, and choose the best action to manage heavily congested traffic and enhance system performance. This article reviews various research papers based on RL models such as Q-Learning, Max pressure intersections, Adaptive learning that can efficiently handle high volume of traffic in urban networks in real time.

Keywords: Traffic Signal Control, Reinforcement Learning, Q-Learning, Max-Pressure, Intersections.

INTRODUCTION

Due to increasing population and increasing mobility, traffic congestion has become a vexing and complex issue in many urban areas. Traditional traffic light managing algorithms manages the green and red lights based on fixed time cycle which is inefficient in urban areas where traffic should be managed based on current traffic situation. Of particular interest are the intersections where traffic bottlenecks are known to occur despite being traditionally signalized [10]. In order to solve the traffic congestion problems many machine learning algorithms have been developed by various researchers. Reinforcement learning (RL), which is one of the machine learning technique has been adopted in Traffic Signal Control (TSC) for monitoring and ameliorating traffic congestion. This method learns from trial and error without making unrealistic assumptions on Traffic Control. RL algorithms have piqued the

attention of researchers and found growing applicability in tackling co-operative TSC challenges [2]. RL is a self-learning AI technique which makes it a suitable approach for handling TSC problems. As RL does not depend on a supervised learning model, which requires a large data set for training, it is widely used by many researchers for solving co-operative TSC problems. RL updates its model continuously with new traffic observations which makes it an efficient algorithm for dynamic traffic control. Many RL based approaches focus on the data transmission delay which is a major issue in a traffic road network. Reinforcement learning algorithms optimize environmental feedback by mapping percepts to actions. These are best suited for domains in which an agent has limited or no previous knowledge. RL algorithms are broadly classified as model-free and model-based. Model-based methods primarily rely on planning, while model-free methods primarily rely on learning. In RL a model-free algorithm (as opposed to a model-based one) can be thought of as an explicit trial-and-error algorithm. Q-Learning is a kind of model-free algorithm which is used to estimate an optimal action selection policy and attempts to improve the policy with no required knowledge on the system. Q-learning is applied for signal light timing to minimize total delay. It is assumed that an intersection behaves similar to an intelligent agent learning to plan according to the current traffic status. Q-Learning does not require a pre-defined model of traffic environment for choosing the actions; it learns the relationships between status, actions and rewards through dynamic interaction with the current traffic control. Moreover, super vision of the learning process is not required for the Q-Learning algorithm which makes it suitable for solving the co-operative TSC problems. In this article, few research papers that applies various types of RL algorithms for solving traffic congestion problem are reviewed. This paper is structured into 5 chapters. Chapter 2 presents the various types of RL algorithms used for controlling TSC. Chapters 3 and 4 review various algorithms that adopt Q-Learning, Max-Pressure, Intersections for solving traffic control signal issues and conclusion is presented in chapter 5.

REINFORCEMENT LEARNING

The RL algorithm improves itself through the learning process of the past. RL is vastly applied in traffic flow control in recent years to learn dynamic traffic behavior. N. Bhave et al. [2] suggested a smart adaptive traffic signal control system based on a single-agent reinforcement learning algorithm model. The model was used with simulation, for a single traffic signal and it showed a substantial decrease in average waiting time for the vehicles at the signal. The suggested system detects real-time traffic situations using object detection and applies reinforcement learning to determine the appropriate green phase timing for that scenario. The suggested system was evaluated in simulation for a single traffic light and demonstrated a significant reduction in average waiting time for cars at the signal. The system implementation cost is far lower which makes it appropriate for deployment in emerging countries. A Traffic light management system based on Deep Reinforcement Learning was developed by J. Luo et al. [13]. The model's input is traffic data which was acquired by vehicle networks or sensors. The state is a collection of vehicle data at the junction. The actions are represented as MDP (Markov Decision Process), and the rewards are the weighted sum of three traffic-related indicators, Cumulative waiting time difference, Queue length, and Cumulative delay. The authors increased the setup of numerous MDP components in the traffic signal control model compared to the previous ATSC (Adaptive Traffic Signal Control) model. This strategy generates a more accurate representation of the traffic situation. The authors improved the settings of several components of MDP and achieved good results and also suggested the research can be extended to consider the cooperative problems in multi-intersections signal control. M. Yu et al. [22] designed a deep deterministic policy Gradient (DDPG) model-based traffic signal management approach with a sensitive structure for each phase. To increase the efficiency of training Deep Reinforcement Learning (DRL) agents, the authors offered two key training techniques: breaking the training process into three parts and the episode break mechanism. They explored the influence of this approach and concluded that the simulation result gives an observation stating that Deep-Q Networks (DQN) based algorithm performs better than DDPG-based algorithm. The efficiency of DQN and DDPG based algorithms were compared using SUMO (Simulation of Urban Mobility) with extensive trials carried out on an isolated crossroads with varying traffic needs.

S. M. A. Shabestary et al. [15] introduced a unique continuous action RL-based cycle-level adaptive traffic signal controller. This controller employs Proximal Policy Optimization (PPO), a cutting-edge RL algorithm, to learn the best technique for providing traffic signals timings during each cycle. The authors stated why they created an

adaptive signal control that plans out the phasing lengths of the next cycle ahead of time rather than making judgments every second by Proximal Policy Optimization – Controller (PPO-C). The system provided the full picture of how the next cycle will look in advance. A reinforcement learning platform was built by S. Touhbiet et al. [18] to adjust traffic signal control to the dynamics of the traffic pattern, which demonstrated robust adaptation and outstanding performance in a real-world junction compared to pre-timed signal control using the Webster technique. The state space represents all the free spaces available in the junction. Furthermore, the new state-space performs well in throughput and average latency, especially for heavy and varied traffic. The analysis of the reward definitions revealed that a reward function's performance depends on the traffic volumes in the intersection and the equipment used to monitor the intersection. The reward function depends on the indicators, such as cumulative delay which require more sophisticated sensors, such as video surveillance or GPS equipped vehicles. A multi-agent decentralized RL algorithm for traffic signal management was designed by C. Wu et al. [19]. Under stochastic traffic flow, variable traffic demand, and information uncertainty, a transfer learning methodology is employed to assess the resilience of the proposed RL-based algorithm and the techniques typically used in practice. The findings revealed that a well-designed RL signal control algorithm outperforms the fixed-time and actuated techniques in online performance. Furthermore, the agent-based system may change its control rules by engaging with an online traffic environment, something rule-based control approaches cannot do. S. Yan et al. [21] introduced a unique way to learn traffic signal controllers using deep reinforcement learning. This method adds a separate equity element to current reward functions. The authors presented an adaptive discounting strategy for conforming to the learning principles of deep reinforcement learning agents and stabilizing training. A simulated and real-world data was used to verify the usefulness of this strategy.

Q-LEARNING

Q-Learning is an unsupervised learning method which is capable of responding through continuous learning and immediate adaptation to the learned techniques. Q-Learning system learns from its past experiences and adapts to make better decisions in future according to the dynamic change of traffic flow in urban traffic network. Instead of learning from large set of training examples, a Q-Learning agent generates its own through the training experiences from the current environment. Various research works based on Q-Learning algorithm is listed below. Abdoos et al. [1] proposed a method for controlling signal lights for each intersection based on Q-learning and extended that approach to 50 intersections. Each intersection is presented as an agent, and the whole system is formed as a multi-agent system. They considered the average queue length in approaching links in a fixed cycle as states of Q-learning. The number of permutations of the approaching links forms the number of states. The proposed method is based on local statistical information gathered in one learning step and tries to learn the best action in different situations. Average queue length in approaching links is used as the statistical information. Jacob et al. [7] used Q-learning to control highway traffic. For isolated intersection, control considers Q-learning with different objective functions. This study seeks to achieve optimal traffic signal control for an isolated intersection. Although there are multiple intersections in an urban traffic network, here the author focused on optimizing traffic signals for one intersection. Q-Learning, was examined for the autonomous, combined and integrated power of freeway, freeway/arterial or express/collector traffic corridor using VMS and ramp metering. The metering rate on the Ramp indication is dynamically changed, based on the action selected by the Q-Learning algorithm. The efficacy of traffic signal timing planner management is the essential answer to traffic congestion. However many of the existing traffic light signal system is not completely optimized based on dynamic traffic circumstances on the route. Y. Chin et al. [18] applied Q- Learning to forecast benefits from future actions and collect rewards from previous experiences. The algorithm improved its judgments for the best potential behaviors and showed good beneficial performance in terms of improving the traffic signal timing plan for dynamic traffic flows inside a traffic network. S. Touhbi, et al. [18] investigated the viability of RL, specifically the application of the Q-learning algorithm for adaptive traffic signal management in various traffic dynamics. Using Paramics, a tiny traffic simulator, A RL control was established for an isolated multi-phase junction. The original aspect of this study is its technique, which employs a new generalized state space with many known reward definitions. The findings of this research demonstrated the benefits of utilizing an RL over fixed signal design, however the effects vary depending on the reward definitions and traffic dynamics analyzed. Jiong Song & Zhao Jin [8] presented a multi-intersection urban traffic light management approach based

on Q-learning. The authors created a reinforcement learning model for the traffic signal control issue and provided an algorithm for determining the best policy. The authors also implemented a simulation environment to illustrate the control impact of this strategy, and the results of the experiments suggested that this method can greatly cut the time for all vehicles to depart the urban traffic network.

MAX-PRESSURE, INTERSECTIONS and ADAPTIVE LEARNING

Boukerche, A. et al. [3] suggested a cooperative traffic signal control strategy based on reinforcement learning to improve the efficiency of cooperative TSC at many crossings. This approach takes into account the traffic conditions at the local junction as well as neighboring intersections. The authors presented a max-pressure-based traffic state representation and a reward model to create an agent that can assess local pressure and pressure from surrounding crossings to prevent superfluous controls of switching traffic signal phases. Furthermore, a traffic status prediction approach for dealing with data transmission delays in vehicular network settings was also suggested. The traffic status prediction system was tested by varying the delay ranges. The findings revealed that the proposed strategy outperformed earlier max-pressure management methods on vehicle-based and intersection-based measures, increasing vehicle demands. The findings proved that the state prediction approach may greatly narrow the gap between real-time and delayed traffic states. This technology showed the potential to handle the data transmission latency problem in automotive networks.

Gong, Y. et al. [6] presented a safety-oriented adaptive signal control method to maximize traffic efficiency and safety at the same time to enhance the traffic safety of the signalized junction. The control agent receives high-resolution real-time traffic data as input and chooses optimal signal phases every second to decrease vehicle delays and the intersection's collision risk. A multi-objective reinforcement learning system employs a double dueling deep neural network as the backend algorithm to handle the discrete optimization issue. One of the single policy multi-objective reinforcement learning algorithms, the weighted sum technique, is used to cope with the trade-off between traffic safety and efficiency. Tang, C. et al. [17] presented and demonstrated an urban traffic route guiding approach with a high adaptive learning capacity and the potential to alleviate congestion. An A^* trajectory rejection approach based on multi-agent reinforcement learning (A*R2) was developed for tackling the congestion problem. The method incorporates both system and user viewpoints to minimize trip time (TT) and travel distance (TD). C. Li et al. [12] used a traffic signal control approach based on the SARSA (State-Action, Reward-State Action) reinforcement learning algorithm. Through the radial base function to approximate value function, and on-line realizing adaptive constructing of state space, the author concluded that the suggested control technique is possible and successful, as it significantly decreases the average delay time for vehicles when compared to the fixed time slice allocation strategy.

CONCLUSION

Traffic signal congestion is a global problem in urban areas which should be addressed by transportation systems to reduce the burden of long waiting time in traffic queues. Various algorithms were designed by researchers for solving congestion problems through synchronizing traffic lights based on current traffic loads. Reinforcement machine learning algorithm does not require an explicit model of the traffic congestion, but can be effectively applied for avoiding congestion by dynamically responding to the frequent changes of traffic flow. From various researches done in this domain, it has been demonstrated that RL algorithms outperform traditional traffic control algorithms. This paper presents a comprehensive review on Reinforcement learning (RL) algorithms addressing TSC problems.

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RESEARCH ARTICLE

A Study to Assess the Effectiveness of Laughter Therapy on Stress among Patients with Acid Peptic Disease at Selected Hospital Cuddalore, Tamilnadu

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ABSTRACT

An quasi experimental study was conducted to assess the effectiveness of laughter therapy on stress among patients with acid peptic disease at Government head quarters hospital cuddalore. A total of 40 subjects who fulfilled the inclusion criteria were selected by using convenient sampling technique. Data were collected through demographic variables and international stress management questionnaire and analyzed through descriptive and inferential statistics. pre-test level of stress were assessed followed by laughter therapy training to the experimental group. The post test level of stress was checked in the interval of 20 days for 3 times. The test accomplishes that the majority of 33 (82.5%) patients had severe stress, 7 (17.5%) patients had moderate stress and none of the patients had mild and no stress respectively in pre test. Whereas 20 (50%) patients had moderate stress and 20 (50%) patients had mild stress on post test 3 in experimental group. In comparison clearly depicts that the 'F' value 118.580 (p value < 0.001) shows that there was a significant reduction in stress level on post test when compared to the pre test.

Keywords: Acid peptic disease, stress, laughter therapy, effectiveness

INTRODUCTION

Acid peptic disease by definition is a disorder of gastric and duodenal mucosal barrier due to hyper or hypo secretion of acid and pepsin into the gastric juice which result in destruction of mucosal layer of stomach and duodenum. Acid peptic disease is one of the commonest disorders encountered in modern society. Factors contributing to peptic ulcer are infectious agent such as helicobacter pylori which spreads through unclean food or water, smoking, alcohol, radiation, increase stress, unhealthy diet, irregular eating habits, physical inactivity, Drugs such as aspirin and pain killers etc. A stress ulcer is not the same as a peptic ulcer that is made worse by stress. While both cause sores in the lining of the stomach and the intestines, a typical peptic ulcer sometimes called a stomach ulcer tends to emerge gradually, as drugs or infections weaken the gastrointestinal lining. Stress ulcers come on suddenly, usually as a result of physiological stress. Stress induces secretion of stomach acid and which causes erosion of gastric mucus membrane. The stomach naturally produces acid to help digest food. When the stomach's acidic environment changes or becomes too acidic, a person may develop symptoms of an ulcer.

STATEMENT OF THE PROBLEM:

A Study To Assess The Effectiveness Of Laughter Therapy On Stress Among Patients With Acid Peptic Disease At Selected Hospital Cuddalore

OBJECTIVES

- To assess the pretest level of stress among patients with acid peptic disease.
- To evaluate the effectiveness of laughter therapy on stress among patients with acid peptic disease.
- To compare the pretest and post test level of stress among patients with acid peptic disease

METHODOLOGY

Quantitative research approach using quasi experimental pretest post test design 40 patients both male and female were chosen by non probability convenient sampling technique who met inclusive criteria in government head quarters hospital cuddalore. The necessary administrative permission was obtained from the hospital authorities. After taking the informed consent, the data were collected by using demographic Performa and pretest level of stress was assessed by international stress management questionnaire. Laughter therapy training was taught for 45 minutes and they were informed to do the same daily and maintain diary. Post test 1 was conducted 20 days after pre test and post test 2 was conducted 40 days after pretest post test 3 was conducted on 60 th day after pretest while coming for follow up. The data collected from subjects were compiled and analyzed by using descriptive statistics such as number, percentage, mean and standard deviation to describe the demographic variables.

RESULTS & DISCUSSION

Result reveals that the majority of 33 (82.5%) patients had severe stress, 7 (17.5%) patients had moderate stress and none of the patients had mild and no stress respectively in pre test. In post test I, majority of 26 (65%) patients had severe stress and 14 (35%) patients had moderate stress. In post test II, majority of 34 (85%) patients had moderate stress and 6 (15%) patients had severe stress. In post test III, 20 (50%) patients had moderate stress and 20 (50%) patients had mild stress. Comparison of stress pain on pre test and posttest shows that the Mean and Standard deviation of pre test was 16.4000 and 2.38371 after the intervention the mean stress level decreases as 14.0500, 10.7750, 4.7500 respectively for post test I, post test II and Post test III. Friedmann's non parametric test has been applied to assess whether the decrease is statistically significant. The significant p value(<0.001) indicates an significant decrease in stress level after the intervention. To get more details on decrease level of stress nemenyi's pair wise test was applied. The significant p value between pre test and post test I (0.008) post test I and post test II (0.002) post test II and post test III (0.002) indicates stress level decreases significantly after intervention. Similar

Study conducted to assess the effectiveness of laughter therapy on stress reduction among the first year nursing students in selected College Tadepalligudem. 30 sample was used for the study. random sampling technique was adopted for the selection of the sample. The result showed that significant reduction in the stress level of nursing students in the experimental group after the introduction of laughter therapy (t= 1.761, df=14, $p<0.05$). The mean percentage of post test stress scores of experimental group (33.66) was less than the post test stress scores of control group (49.3).The study conclude that laughter therapy were effective in reducing stress among nursing students.

CONCLUSION

Assessed the effectiveness of laughter therapy on stress among patients with acid peptic disease. The study revealed that the majority of 33 (82.5%) patients had severe stress, 7 (17.5%) patients had moderate stress and none of the patients had mild and no stress respectively in pre test. Whereas 20 (50%) patients had moderate stress and 20 (50%) patients had mild stress on post test 3 in experimental group. In comparison clearly depicts that the 'F' value 118.580 (p value < 0.001) shows that there was a significant reduction in stress level on post test when compared to the pre test.

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Table 1. Demographic Characteristics of the subjects

Variables	No.	%
Age in years		
25-30	3	--
31-35	6	10.0
36-40	15	40.0
Above 40	16	50.0
Gender		
Male	24	60.0
Female	16	40.0
Residence		
Rural	10	25.0

Urban	19	47.5
Semi urban	11	27.5
Religion		
Hindu	30	75.0
Christian	8	20.0
Muslim	2	5.0
Others, specify	--	-
Highest Educational status		
Illiterate	3	7.5
Schooling	32	80.0
Collegiate	5	12.5
Others	0	--
Occupational status		
Unemployed	14	35.0
Self employed	13	32.5
Manual labour	10	25.0
Clerical	3	7.5
Technical / professional	0	--

Table 2. Socio-economic status and other background variables of the subjects

Variables	No	%
Family income per month in Rupees		
a)≥199862	1	2.5
b)99931 – 199861	0	0
c)74756 -99930	3	7.5
d)49962 -74755	7	17.5
e)29973-49961	5	12.5
f)10002-29972	8	20
g)≤ 10001	16	40
(As per Kuppusamy scale – socioeconomic 2020)		
Class of family		
Upper (I)	2	5.0
Upper middle (II)	0	0
Lower middle (III)	3	7.5
Upper lower (IV)	13	32.5
Lower (V)	22	55.0
Dietary pattern		
Vegetarian	3	7.5
Non vegetarian	6	15.0
Mixed	8	20.0
Vegan	8	20.0
Too spicy	15	37.5

Source of getting health information		
Friends	3	7.5
Relatives	9	22.5
Health personnel	20	50
Mass media	8	20
Any other specify	0	0

Table .3. Effectiveness of experimental group on Stress score

Assessment	Mean	SD	Friedmann's test result		Nemenyi's (pair wise) test result	
			F-value	P-value	Comparisons	P-value
Pre test	16.4000	2.38371	118.580	<0.001	Pre-post1	0.008 **(S)**
Post test1	14.0500	2.35285			Post1-post2	0.002 **(S)**
Post test2	10.7750	2.42305			Post2-post3	0.002 (**S**)
Post test3	4.7500	1.48064				

(S – Significant, NS – Not Significant)

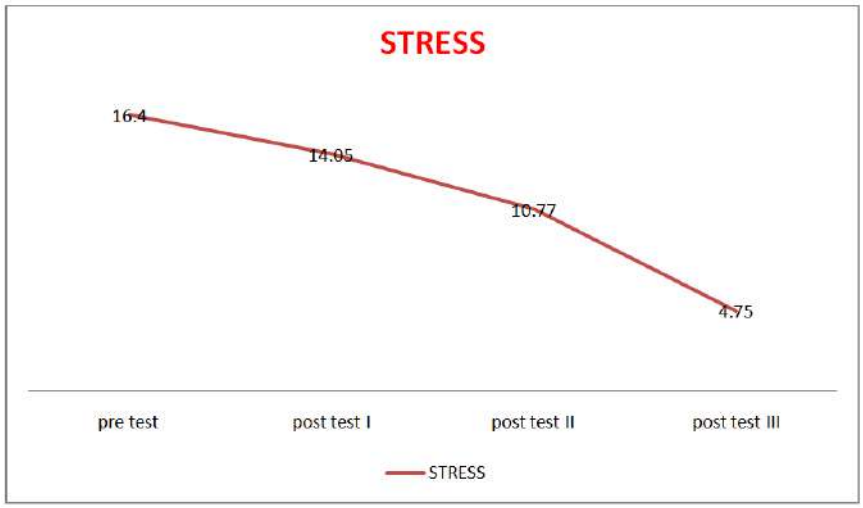

Fig 1. Comparison of mean Pre test vs post test I, post test I vs Post test II and Post test II vs Post test III level of Stress

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RESEARCH ARTICLE

Effect of Foliar Fertilization of Different Chemicals on Rainfed Pearl Millet

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ABSTRACT

Field Experiment was conducted during June-September-2018 with an objective to enhance growth, yield, nutrient uptake and post harvest nutrient status of rainfed Pearlmillet through foliar fertilization. The experiment was laid out in randomized block design with seven treatments and replicated thrice. The treatment details includes, T1 – Control (Farmer practice), T2 – Full RDF, T3 - 50% RDF and foliar spraying of "Revive plus" @ 2 g litre-1 twice at 25 and 50 DAS,T4 - 50% RDF and foliar spraying of "Revive plus" @ 3g litre-1 twice at 25 and 50 DAS,T5 - 50% RDF and foliar spraying of "1% KCl" twice at 25 and 50 DAS, T6 – 50% RDF and foliar spraying of "100ppm Salicylic acid" twice at 25 and 50 DAS, T7 - 50% RDF and foliar spraying of "Orthosilicic acid" @ 2ml litre-1 twice at 25 and 50 DAS. The results of the experiments revealed that foliar application of different chemicals significantly influenced the growth and yield attributes and yield, nutrient uptake and post harvest nutrient status of cumbu. Among the various treatments tried, T4 - "Revive plus" @ 3g litre-1 twice at 25 and 50 DAS recorded maximum values for growth and yield attributes viz., plant height, LAI,DMP, root length and chlorophyll content and yield attributes viz., number of earheads plant-1, number of grains earhead-1, test weight, seed yield and stover yield ,harvest index and economics .This treatment also registered maximum values for NPK uptake and post harvest nutrient status of pearl millet. The lowest values for growth and yield attributes and yield were recorded in T1 – Control treatment.

Keywords: Rainfed Pearlmillet, plant height, LAI,DMP, harvest index and economics, twice at 25 and 50 DAS.

INTRODUCTION

Drought has been a recurring feature of agriculture in India. In the past, India experienced twenty four large scale droughts in with increasing frequencies during the periods 1891-1920,1965-1990 and 1999- 2012 (NRAA, 2013). Long-term rainfall data for India indicates that rainfed areas experience 3-4 drought years in every 10 year period. Of these, two to three are in moderate and one or two may be of severe intensity (Srinivsa Rao *et al.,* 2013). Occurrence of the drought is very frequent in the meteorological sub divisions like West Rajasthan, Tamilnadu, Jammu & Kashmir and Telengana (NRAA, 2013). The risk involved in successful cultivation of crops depends on the nature of drought (chronic and contingent), its duration, and frequency of occurrence within the season. The term rainfed agriculture is used to describe farming practices that solely depend on rainfall for water. It provides much of the food consumed by poor communities in developing countries. Rainfed crop cover more than 80% of global cropped area and account for 60-70% of global crop production, but production is frequently limited by drought and soil moisture stress (Wood *et al.,* 2000). In India around 65-70% of cultivated lands coming under rainfed drylands and they contributing about 45% of nation's food grain production. Most of the crops belongs to millets (>95%), oilseeds (90%) and pulses (90-95%) are cultivated on these lands with low productivity. The reasons attributed to low productivity may be inadequate rainfall, poor soil fertility, occurrence of dry spells drought during cropping season, high temperature, more PET than rainfall etc.,. Of these drought at critical period crops season drastically reduces the crop yield. The effect of water stress was found to decrease significantly in photosynthetic rate (Hejank, 2003) ,transpiration rate (Gupta *et al.,* 2003), chlorophyll stability index, stomata conductance and relative water content (Gupta *et al.,* 2003). Crops such as millet, foxtail, sorghum and cowpea are also grown in the arid and semi-arid areas affected by drought stress at the reproductive stage (Emam and Zavorakh, 2004). The All India coordinated Research Project for Dryland Agriculture (AICRPDA) in association with state Agricultural universities, Technical Universities and Research Institutes of Indian council of Agricultural Research have developed location-specific rainfed technologies to cope with different drought situations. The key technologies for drought mitigation are in situ moisture conservation, rainwater harvesting and recycling, resilient crops and cropping systems including contingency crop plant, foliar spraying, integrated farming system etc., (Srinivasa Rao *et al.,* 2014). Among these dryland technologies foliar spraying of nutrients and anti-transpirants found to be effective in mitigating drought moisture stress during cropping seasons.

MATERIALS AND METHODS

The Field Experiments were conducted during June-September-2018 at the farmer's field at Kunjaram village in Ulundurpet Taluk of Villupuram District, Tamil Nadu. The experimental site is situated at 11°41′26″N latitude, 79°17′30″E longitude with an altitude of 66 m above mean sea level. The texture of the experimental field soil is sandy loam which is low in available nitrogen, high in available phosphorus and high in available potassium content. Pearlmillet CO9 variety was chosen for this study. The experiment consisted of seven treatments and was laid out in Randomized Block Design with three replications. The treatments imposed in the experiment with different chemicals viz., T1- Control (Farmer's practice), T2 **-** RDF 40:20:0 Kgs of NPK ha-1, T3 - 50% RDF and foliar spraying of Revive plus @ 2g litre-1 at 25 DAS and 50 DAS, T4 **-**50% RDF and foliar spraying of Revive plus @ 3g litre-1 at 25 DAS and 50 DAS, T5 **-**50% RDF and foliar spraying of 1% KCl at 25 DAS and 50 DAS, T6 **-**50% RDF and foliar spraying of 100ppm Salicylic acid at 25 DAS and 50 DAS and T7 -50% RDF and foliar spraying of Orthosilicic acid @ 2ml litre-1 at 25 DAS and 50 DAS. The recommended dose of 40:20:0 kgs of NPK ha-1 for pearl millet varieties were applied in the form the urea (46 % N), DAP (18 % N and 46 % P2O5) respectively. Fertilizers were applied fully as basal.

RESULTS AND DISCUSSION

Growth attributes (Table 1)

Among the various foliar nutrition, foliar spray of Revive plus @ 3g litre-1 (T4) registered the higher plant height, LAI, DMP, chlorophyll content and root length of sorghum and it was comparable with foliar spraying of Revive plus @ 2g litre-1 (T3) but superior to other foliar fertilization. Increased plant height under this treatment might be due to balanced and increased availability of nutrients to the crop due to foliar application of nutrients. The increased chlorophyll content might be due to the fact that of nitrogen is a constituent of chlorophyll molecule which is expected during rapid grain filling leaves. These results are in agreement with the findings of by Reddy *et al,* (2018). Increased leaf area index and dry matter production might be due to increased availability of nutrients and its uptake by the crop resulted in higher growth attributing characters which reflected in higher yield of sorghum. These results are in close conformity with the findings of Lakhan singh *et al*, (2017).

Yield attributes and yield (Table 2)

Among the various foliar nutrition tried, foliar spray of Revive plus @ 3g litre-1 (T4) recorded the maximum number of earhead plant-1, number of grains earhead-1, test weight, grain yield, stover yield and harvest index for pearlmillet and sorghum. It is comparable with foliar spraying of Revive plus @ 2g litre-1 (T3) but superior to other foliar fertilization. The combined fertilizer application through foliar nutrition increased yield attributes and yield of millets might be due to the supplementation of plants with the three major nutrients and micronutrients together and these elements enhancing and including most of metabolic processes, N increased the protein formation, phosphorous in the formation of nucleic acids and energy compounds while potassium affected the water adjustment and carbohydrate transportation. This was in line with the findings of Hussein *et al.* (2011). Grain yield increased by foliar application of potassium due to improving the enzymes activity in the plant, which leads to easy translocation of photosynthates from leaf to grain in maize. Similar finding was earlier reported by Abid Ali *et al.* (2016). Application of nutrients influenced the higher growth parameters and improves drymatter accumulation which in turn improves yield parameters. Later this drymatter was translocated to different yield components thereby increases grain yield. This was evidenced through the studies of Shankar *et al,* (2017) in little millet. The harvest index was increased by the foliar application of nutrients might be due to the nutrients are utilized for increasing the vegetative growth than producing economic yield. The results of present study fall in line with the findings of Lakhan singh *et al.,* (2017) who reported that increased availability of nutrients enhanced growth and yield attributing characters which reflected in higher yield of pearlmillet.

Nutrient Uptake (Table 3)

Nutrient uptake of cumbu were significantly influenced through foliar fertilisation of different chemicals. Nutrient status is an important and deciding factor in judging the total dry matter accumulation in plants. Among the different treatments tried, maximum N,P and K uptake was registered under foliar spray of revive plus @3 g/litre over rest of treatments. Application of nutrients enhanced the N.P and K uptake. This might be due to increased DMP, easily available and rapid absorbtion of nutrients by the crop and higher dry matter production could be attributed to higher nutrient uptake. Similar result was documented by kavya *et al*.,(2009).

POST HARVEST NUTRIENT STATUS (Table 3)

Foliar nutrition of different chemicals on post harvest nutrient status of pearl millet was significant. among the different chemicals tried, control treatment recorded the maximum values for post harvest available nutrient status in cumbu. This might be due to poor nutrient uptake compared to rest of the treatments. Similar trend of result was earlier reported by senthil *et al*.,(2003).

ECONOMICS (Table 3)

Among the various foliar nutrition, foliar spray of Revive plus @ 3 g litre-1 (T4) recorded maximum net return Rs.31296.26 and BCR value 2.04 over other foliar application. This might be due to higher seed yield and market

value of the produce. This was followed by foliar spraying of Revive plus @ 2 g litre-1 (T3).The least value was recorded in control (T1) in pearl millet.

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Table1: Effect of foliar fertilization of different chemical on growth attributes of pearl millet Treatments

	Plant height(cm) At harvest stage	LAI at flowering stage	DMP (Kg ha-1) at harvest stage	Chlorophyll content (mg g-1) at flowering stage	Root length (cm) at harvest stage
T1	180.2	3.42	6601.24	44.54	25.9
T2	185.3	3.78	6745.98	48.44	27.1
T3	193.3	3.98	6988.65	53.38	28.1
T4	194.9	4.08	7024.65	54.47	28.4
T5	188.5	3.89	6803.47	50.78	27.3
T6	183.4	3.54	6687.24	46.54	26.4
T7	190.4	3.92	6880.12	51.36	27.6
SE.d	**3.40**	**0.06**	**119.99**	**0.87**	**0.56**
CD (p=0.05)	**7.42**	**0.13**	**261.44**	**1.89**	**1.22**

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REVIEW ARTICLE

A Study to Assess the Level of Mental Health and Self-Esteem among School Children in Selected Schools at Puducherry

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ABSTRACT

A descriptive study was conducted to assess the level of mental health and self-esteem among school children in selected school, at Puducherry. A total of 100 study participants who fulfils the inclusion criteria were selected by non-probability purposive sampling technique in St.Joseph English high school at Thavalakuppam, Puducherry. The data were collected through demographic variables, Strength and difficulty questionnaire and Rosenberg self esteem scale and analyzed through descriptive & inferential statistics. The result reveals that 61(61%) had borderline mental health, 36(36%) had abnormal mental health, 3(3%) had normal mental health and 27(27%) had low self-esteem and 73(73%) had Normal self-esteem. Correlation value of r = -0.406 shows a moderate positive correlation between mental health status and self – esteem among school children which clearly infers that when the mental health of the school children improves/increases their self-esteem also increases.

Keywords: Descriptive, Mental health, Self-esteem, School children

INTRODUCTION

"Children must be taught how to think, not what to think" - Margaret Mead
"One teacher can shape a child
One child can shape the world"

School age is the beginning of a person's life. School plays an important function in human life. Any individual's personality development requires the help of family and school. In human existence, school is regarded as the primary external environment. Exposure in the classroom has a direct impact on a child's development. School provides a learning environment in which students can acclimatise to a group of people, engage with them, and attain various goals. School has a significant impact on a child's personality development [1],[2]. Subjective well-being, perceived self-efficacy, autonomy competence, intergenerational reliance, and self-actualization of one's intellectual and emotional potential are only a few examples of mental health. Mental health is beneficial to academic performance. Academic performance, classroom behaviour, and learning engagement are all improved in children with good social emotional mental health [3]. Self-esteem relates to an individual's perception of their own abilities and limits. Self-esteem is defined as a person's feeling good about oneself. Individuals with high self-esteem treat themselves and others with respect [4]. This is where mental wellness and personality growth begin. Children's good self-esteem is produced by effective psychosocial development [4, 5]. According to Erickson's view, a child's unmet needs throughout the school years contribute to low self-esteem and other difficulties. Children's self-esteem is important for their mental wellness [6].

NEED FOR THE STUDY

According to the United Nations, children and adolescents spend an average of 10 to 15 years in education around the world. Given the length of time spent in school, it is necessary to build good behaviours. It also promotes children's health and well-being from an early age. Stressors, anxiety, traumas, abuse, learning impairments, and even bullying in children show a lack of discipline formulation. As a result, these habits have proven to make a difference in both their professional and personal lives. [7]. According to studies, the epidemiological age of most mental health illnesses in people began in childhood and adolescence. According to DSM-IV-TR criteria, about 7.5 percent of teenagers have one or more mental health disorders [8]. In 2014, 6.1 million youngsters worldwide were out of school. Out of every 100 pupils, 29% of both girls and boys drop out before completing the elementary school cycle. (SRI-IMRB surveys from 2009 and 2014) [9]. Half of primary school students, or over 50 million youngsters, are not performing at grade level. (Source: NCERT 2017 National Achievement Survey) [10] In Mangalore, South India, an experimental study was conducted to identify behavioural problems among school pupils (2021). The results revealed that 8.7% of the students (65 out of 750) had behavioural issues that their instructor had identified**.** Low self-esteem creates poor academic performance, depression, anxiety, learning problems conduct disorder, behavioral problems and personality problems. Some of the psychosocial issues may be unable to reveal and addressed. These leads to poor interpersonal relationship, low peer relationship, substance abuse and aggressiveness [11].

STATEMENT OF THE PROBLEM

A study to assess the level of mental health and self-esteem among school children in selected schools at Puducherry.

OBJECTIVES:

- To assess the level of Mental health and Self-esteem among school children.
- To find out the Correlation between the level of mental health and self-esteem among school children
- To find out the association on level of Mental Health and Self-esteem among school children with selected demographic variables.

DELIMITATION

- The school children for the age group between 9-12 yrs
- The study is limited to 100 school children

- The duration of the study is delimited to 4 weeks

RESEARCH METHODLOLOGY

Research Approach - Quantitative research approach
Research Design – Descriptive with survey research design.
Setting- St Joseph English high School, Thavalakuppam, Puducherry
Population - Both male and female school children who were willing to participate in the study.
Sample –Both male and female school children who are residing in and around Thavalakuppam, Puducherry, who fulfills the inclusion criteria.
Sample size - 100 male and female school children.
Sampling Technique - Non probability purposive sampling technique
Criteria for sample selection

Inclusion Criteria:
Children those who
- willing to participate in this study
- those can know write and understand Tamil and English
- present on the day of data collection

Exclusion Criteria
Children those who
- sick on the day of data collection
- any physical and mental illness
- who are less than age of 09 yrs and more than 12 yrs.

DESCRIPTION OF THE TOOL
The tool used for data collection was an interview technique. It consists of two parts:

Part I - Demographic Data (age ,gender, parents educational status, parents occupational status, income, religion, family system, location of family, health status)

Part II – Strength and difficulty questionarrie
The Strengths and Difficulties Questionnaire (SDQ) is a brief, 25-item, measure of behavioural and emotional difficulties that can be used to assess mental health problems in children and young people aged 4–17 years. The SDQ queries positive and negative attributes displayed by the child in the past 6 months across five subscales: Emotional Symptoms (e.g., often unhappy, downhearted), Conduct Problems (e.g., fights with other children), Hyperactivity/Inattention (e.g., constantly fidgeting or squirming), Peer Relationship Problems (e.g., tends to play alone) and Prosocial Behavior (e.g., considerate of other people's feelings).

Part III – Rosenberg self-esteem scale
Rosenberghself esteem scale, A 10-item scale that measures global self-worth by measuring both positive and negative feelings about the self. The scale is believed to be uni-dimensional. All items are answered using a 4-point Likert scale format ranging from strongly agree to strongly disagree. It is related to overall feelings of self-worth or self-acceptance.

SCORING And INTERPRETATION
Strengths and Difficulties Questionnaire (SDQ)
The score for every statement had the options of three responses:

Rosenberg self-esteem scale

Items 2, 5, 6, 8, 9 are reverse scored. Give "Strongly Disagree" 1 point, "Disagree" 2 points, "Agree" 3 points, and "Strongly Agree" 4 points. Sum scores for all ten items. Keep scores on a continuous scale. Higher scores indicate higher self-esteem.

DATA COLLECTION PROCEDURE

- Prior to data collection a **formal written permission** was obtained from the concerned school authorities of the selected school at Puducherry.
- A written **informed consent** was obtained from the parents or guardian and teachers of the sample with assurance of confidentiality.
- **All participants were informed** about the study
- **100 study participants** who met the inclusion criteria were selected by using purposive sampling technique.
- The researcher **collected the baseline information through the demographic variables** and the level of **mental health and self-esteem** was assessed by using the SDQ questionnaire and Rosenberg self-esteem scale was used.

DATA ANALYSIS & INTERPRETATIONS

ORGANISATION AND PRESENTATION OF DATA: The analysis of data was organized and presented under the following sections.

- **SECTION A:** Distribution of the study participants by their demographic variables
- **SECTION B:** Frequency and percentage distribution of level of mental health and self-esteemamong school children.
- **SECTION C:**Correlation between the level of mental health and self-esteem among school children
- **SECTION D:**Association between the level of mental health and self-esteem among school children with selected demographic variables

FREQUENCY AND PERCENTAGE DISTRIBUTION OF DEMOGRAPHIC VARIABLES OF SCHOOL CHILDREN

Most of the school children, 65(65%) were aged between 11 – 12 years, 57(57%) were male, 44(44%) of parents were educated upto to 9 – 12thstd, 44(44%) of parents had salaried worker, 58(58%) had monthly income of <Rs.5000, 69(69%) were Hindus, 85(85%) belonged to nuclear family, 51(51%) were residing in urban area, 68(68%) were married and 73(73%) had healthy health status. Pie diagram depicts that among school children, 61(61%) had borderline mental health, 36(36%) had abnormal mental health and 3(3%) had normal mental health.

The Bar diagram shows that among school children, 27(27%) had low self-esteem and 73(73%) had Normal self-esteem. The scatter diagram shows that the mean score of mental health among school children was 27.68±6.12 and the mean score of self-esteem was 19.40±4.12. The calculated Karl Pearson's Correlation value of r = -0.406 shows a moderate positive correlation between mental health status and self – esteem among school children which clearly infers that when the mental health of the school children improves/increases their self-esteem also increases. The table shows that the demographic variables did not show statistically significant association with level of mental health and self-esteem among school children.

DISCUSSION

OBJECIVES	RESEARCH FINDINGS	SUPPORTIVE STUDIES
Assess the level of Mental health and Self-esteem among school children.	The study findings reveals that 61(61%) had borderline mental health, 36(36%) had abnormal mental health, 3(3%) had normal mental health and 27(27%) had low self-esteem and 73(73%) had normal self-esteem.	At least one sort of social isolation is experienced by 27.5 percent of Danish high school pupils. Mental health problems (depression symptoms, anxiety symptoms, stress, sleep problems, suicide ideation, non-suicidal self-injury, eating disorder, body dissatisfaction, and low self-esteem) were positively linked with social disconnectedness, while mental well-being was negatively associated.
Find out the Correlation between the level of mental health and self-esteem among school children	Correlation value of r = -0.406 shows a moderate positive correlation between mental health status and self – esteem among school children which clearly infers that when the mental health of the school children improves/increases their self-esteem also increases.	In order to reduce current and future mental health problems and promote wellbeing, research shows that school belonging initiatives are necessary.
Find out the association on level of Mental Health and Self-esteem among school children with selected demographic variables.	The selected demographic variables did not show statistically significant association with level of mental health and self-esteem among school children.	None of the research investigations corroborated the findings.

IMPLICATIONS FOR NURSING

NURSING EDUCATION	Nurse educators can incorporate in Nursing curriculum regarding mental health and self-esteem of school children for the early identification of abnormalities and for the prompt referral.
NURSING SERVICE	Health care professionals can conduct soft skill and life skill trainings to upgrade the mental health and self-esteem of school children.
NURSING ADMINISTRATION	Nursing students and Nurses can organize various programs to promote awareness among parents and teachersfor the betterment of school children regarding their mental health and self-esteem.
NURSING RESEARCH	Nurse researchers can able to create innovative strategies for the children to improve their mental well-being and attain high self-esteem to enhance their quality of life throughout their lifespan.

RECOMMENDATIONS

Based on the study findings the following recommendations have been made for the further study.

- A comparative study can be conducted between the adolescent and early adulthood.
- A study can be conducted using life skill training programme among school children, teachers and parents.
- A study can be conducted for large sample to generalize the research findings.

CONCLUSION

The present study findings concluded that, when the mental health of the school children improves significantly their self-esteem also increases.

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Table 1.

not true	0
somewhat true	1
certainly true	2

Table 2.

SDQ scoring	Normal	Borderline	Abnormal
Total difficulty score	0-15	16-29	30-50
Emotional symptoms score	0-5	6	7-10
Conduct problems score	0-3	4	5-10
Hyperactivity score	0-5	6	7-10
Peer problems score	0-3	4-5	6-10
Prosocial score	6-10	5	0-4
Total impact score	0	1	≥2

Table 3. Rosenberg self-esteem scale

TOTAL SCORE	SELF ESTEEM
15 & ABOVE	Normal
<15	Low self esteem

Table 4. Association of Mental Health and Self Esteem among School Children with Their Selected Demographic Variables

N= 100

Demographic Variables	Mental Health	Self-Esteem
	One Way ANOVA / Unpaired "t test Value	One Way ANOVA / Unpaired "t test Value
Age	F=0.038 p=0.962 N.S	F=0.327 p=0.722 N.S
9– 11		
11 – 12		
>12		
Gender	t=0.041 p=0.867 N.S	t=0.647 p=0.519 N.S
Male		
Female		
Parents education	F=0.423 p=0.737 N.S	F=0.259 p=0.855 N.S
Illiterate		
1 – 8thstd		
9 – 12thstd		
Graduate / Post Graduate		
Parents occupation	F=0.749 p=0.589 N.S	F=0.415 p=0.837 N.S
Not working		
Self employed		
Agriculture		
Salaried worker		
Business		
Government employee		
Homemaker		
Monthly income	F=0.018 p=0.982 N.S	F=0.467 p=0.628 N.S
<Rs.5000		
Rs.5001 – 10000/-		
Rs.10001 – 20000/-		
>Rs.20000/-		
Type of family	t=0.663 p=0.513 N.S	t=0.885 p=0.384 N.S
Nuclear family		
Joint family		
Residence	F=0.491 p=0.613 N.S	F=0.960 p=0.387 N.S
Rural area		
Semi-urban area		
Urban area		
Marital status	F=0.578 p=0.631	F=0.209 p=0.890
Married		

Demographic Variables	Mental Health	Self-Esteem
	One Way ANOVA / Unpaired "t test Value	One Way ANOVA / Unpaired "t test Value
Unmarried	N.S	N.S
Widow/Widower		
Divorced		
Health status	t=0.497	t=0.745
Healthy	p=0.621	p=0.460
Unhealthy	N.S	N.S

N.S – Not Significant, p>0.05

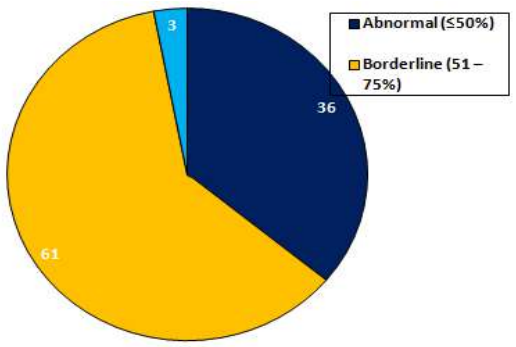

Fig.1. Percentage distribution of level of mental health among school children

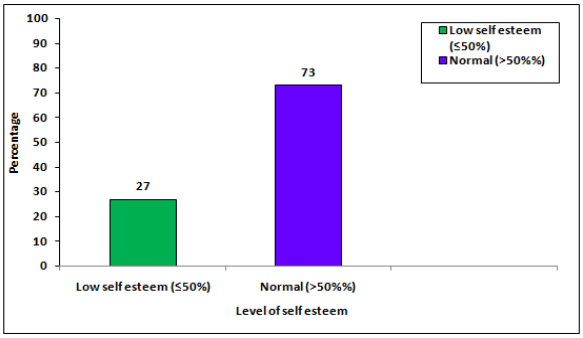

Fig. 2. Percentage distribution of level of self-esteem among school children

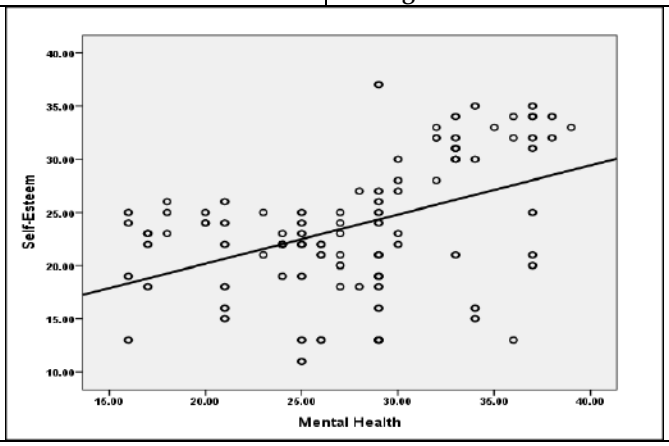

Fig.3. Scatter Plot Diagram showing the correlation between the level of mental health and self-esteem among school children

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RESEARCH ARTICLE

Interrogating Dilemma in Nayomi Munaweera's *What Lies Between Us*

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ABSTRACT

Molestation is one of the evil activities that give the victim a trauma for a long time; sometimes for the whole life. Situations get bitter when the victim is a child and is abused by his/ her close one. It not only gives physical anguish but also mental agony. Sometimes the trauma results in post traumatic socio disorder that compels the victim to lead an imbalanced life in dilemma. Nayomi Munaweera's *What Lies Between Us* is an account of psychological destruction due to childhood trauma and the despondency and agony of a devastated young unmarried woman that headed on a path to destruction. It is a confession of a distressed mother who performs a reprehensible offense. The paper intends to explore psychological reasons behind the protagonist's unusual action in the light of Alfred Adler's 'Theory of Individual Psychology'. Although the protagonist has a happy family but her flaw and secret continue to flake her life. Her childhood abuse continues to haunt, her past and present bump into the extent that she kills her own daughter.

Keywords: Dilemma, Motherhood, Psychology, Relationships, Vacillation.

INTRODUCTION

"I wonder what it would have meant if I could have spoken up in my childhood. What would it have meant if I knew I would have been believed? This is not a justification. This is only my truth."

Nayomi Munaweera's *What Lies Between Us* (2016) is a novel that explicates a tragic chain of circumstances which leads a young mother to ultimate failure. Munaweera vividly portrays the haunting testimony to the destructive

power of childhood trauma and records unnamable sin committed by the narrator. Munaweera unfolds the story of *What Lies Between Us* through the mouth of an unnamed narrator who unveils herself at the last of the novel when she notifies herself - Ganga. Using the plot device of flashback where Ganga narrated her story from her childhood to present, Munawerra showcases the unreliability of memory. Melissa R. Sipin states, "*What Lies Between Us* ties the personal violation of the body — molestation. It is a book that unravels how the child-abused protects herself, how she becomes the star immigrant daughter who achieves the American dream, but also how the past cannot be driven from her body even in a new, foreign land, how, again, trauma sinks into the bones and propagates in the life of the abused-child-turned-mother."

Set in Sri Lanka, the story revolves around Ganga, the daughter of affluent Sinhalese parents, who grows up in a huge but too empty house for a single child. Her mother, an unpredictable woman; suffering from an impairing depressive disorder and father, a well known university professor but a moody alcoholic do not shower required love on her. The girl's only companion and friend is Samson, the gardener. Turning teen teaches her to suppress as well as hide her emotions. The abuse that she is experiencing from the day after her 11th birthday when she is molested not only ordeals her physically but also torments her mentally and she slips into postpartum psychosis. She deems that this evil activity is practiced by Samson but she does not disclose it to her parents. Death of her father shatters her completely. She, with her mother migrates to America to live with her mother's sister. Here, she enjoys the liberty and freedom from the evil practice of molestation but her childhood abuse torments her now and then. Falling in love and marrying Daniel though gives some comfort and sense of completeness to Ganga but her childhood abuse does not let her lead a healthy life that resulted that her husband and family slowly abandon her and take away her only daughter with them. When Ganga's mother reveals the heartbroken truth about her childhood abuse she takes a decision which is very extraordinary hard for a mother to execute.

Maltreatment during childhood has far reaching consequences in adulthood such as inability to trust, low self esteem, depression, relationship problems, sexual problems, learning difficulties, eating disorders and alcohol or drug problems (Berns, *Child, Family, School, Community Socialization and Support* 167). Child Sexual Abuse is a widespread problem that affects both the current and future well being of victims. Throughout their lives child survivors of sexual abuse are at high risk for anxiety, inappropriate sexual behavior, anger, guilt, shame, depression, Post Traumatic Stress Disorder (PTSD) and many other emotional and behavioral problems. Inferiority feeling, particularly due early experiences of humiliation ensue adverse behaviour in the sufferers that results in vacillation in relationships and impels them to live in dilemma which creates hurdles for a cherished life. "Child Sexual Abuse affects more than 1 out of 5 women and one out of 10 men worldwide" (Collin-Vézina et al).

The World Health Organization (WHO) defines child sexual abuse as:

The involvement of a child in sexual activity that he or she does not fully comprehend, is unable to give informed consent to, or for which the child is not developmentally prepared and cannot give consent, or that violate the laws or social taboos of society. Child sexual abuse is evidenced by this activity between a child and an adult or another child who by age or development is in a relationship of responsibility, trust or power, the activity being intended to gratify or satisfy the needs of the other person. This may include but is not limited to: the inducement or coercion of a child to engage in any unlawful sexual activity; the exploitative use of child in prostitution or other unlawful sexual practices; the exploitative use of children in pornographic performances and materials. ("Report of the consultation on child abuse prevention")

Alfred Adler created theory of 'Individual Psychology' in the early 1900s to understand individual in their social milieu. Unlike Sigmund Freud, he put emphasis that an individual cannot be properly figured out as a set of parts but has to be studied as a whole. "Individual Psychology answers the question 'How is it possible for one individual to influence the behaviour of another?' by saying that this phenomenon is one of the accompanying manifestations of our psychic life" (62). It is the theory that examines the latent psychological issues. Richard E. Watts opines "Adlerian theory affirms that humans construct, manufacture, or narratize ways of viewing and experiencing the

world and then takes these fictions for truth" (125). Further he asserts "An integration of cognitive, existential-humanistic, psychodynamic, and systemic perspectives, Adlerian theory is a holistic, phenomenological, socially-oriented, and teleological (goal-directed) approach to understanding and working with people. It emphasizes the proactive, form-giving and fictional nature of human cognition and its role in constructing the "realities" that persons know and to which they respond"(125). Thus, Adlerian theory examines development and growth of children which deems that a dispirited child misbehaves and experiences inferiority feeling if early experiences of humiliation results in adverse behaviour. Moreover, Adlerian theory focuses that instead of parts entire individual should be analysed. He also avows that family strongly influences the personality.

A feeling of human connectivity and a zeal for individual's development are the main features of mental health. When underdeveloped, inferiority may haunt an individual which outcomes in a self-centered, emotionally or materially exploitive figure. Adler states, "We have been able to determine that the impressions which storm in upon every individual from the earliest days of his infancy influence his attitude throughout his whole life" (*Understanding Human Nature* 42). Ganga, the sufferer of lack of affection from her mother and molestation from her father undergoes an abnormal mental health and has to put up with PTSD (Post Traumatic Socio Disorder) She neither could become a groomed woman, good daughter or wife nor could become a good mother. "Aside from post-traumatic stress and dissociation symptoms, a significant number of other mental health and behavioral disturbances have been linked to Child Sexual Abuse" (Collin-Vézina et al).

Annie Zaidi in her review "Inheritance of Loss" on *What Lies Between Us* comments "This is a book about trauma, intergenerational and colonial trauma, but it is also about the sacrificial monstrosity of motherhood. It is a story that unfolds how trauma sinks into the bones and repeats itself, propagates, how it becomes parasitic" (*The Hindu*). The paper tries to explore the latent fear which leads the protagonist into a traumatic dilemma which results in her unpredictable and uncommon behaviour due to her peeping, horrific decisive past.

Mother-daughter Relationship is not only stronger, intimate, and affectionate but it has its challenges also. This is the relationship that facilitates a woman to establish every other relationship in her life. Mother is the one who "denies herself, mortifies her flesh, suffers in silence rather than let her child feel the smallest discomfort. All creatures abide by this law. This is the way of nature. To be otherwise is to be unnatural, to be monster, outside the pole." (Prologue *What Lies Between Us*). When the mother shows detachment towards her daughter, it causes impassiveness in the relationship and leaves emotional scars. Rosjke Hasseldine states, "Mothers and daughters frequently tell me that they feel ashamed about their relationship difficulties. They feel that they "should" be able to get along because popular wisdom tells them that mothers and daughters are supposed to be close. This societal expectation makes mothers and daughters to blame themselves for causing their relationship difficulties" (*Counseling Today* 46). Non-sharing of emotions, problems and difficulties are some of the reasons for imbalanced relationship between Ganga and her mother. During her childhood Ganga was the witness of her mother's abnormal behaviour. She says "I go and sit against door, my knees folded under me, an ear pressed to the wood. I hear nothing… For hours I wait to hear the slightest sound, the merest whisper of evidence that she is inside. Crying, shouting, raging- anything rather than this haunted silence" (24). This outer silence was creating volcano in the heart and brain of the child. She asserts "This is the bane of childhood, isn't it? That the small person is entirely powerless, entirely dependent on the large person despite whatever grace the larger might or might not possess" (272). Throughout her childhood helpless and inferior Ganga craved for her mother's love. After her father's death immigrant adolescent Ganga desired the empathy of her the most but she her mother never showed which Ganga portrays by saying "She hasn't looked at me closely since we lost Thata. She has looked at everything else, but not at me" (98). Ganga could never share her emotions and sexual trauma with her mother. Hans W. Loewald says "Repression is a throwback to that older plane of experiencing: undesirable and unacceptable memories, thoughts, fantasies, by being excluded from ego organization, sink back to that raw form of mentation which is conceptualized as the dynamic unconscious or id" (*Psychoanalysis and the History of the Individual* 12). She used to see the images of her drowned father and missed Samson that used to perturb her mental peace but she could never disclose to her mother and says, "The ghost of one lost man rises from the water. The shadow of that other lost man waits on the bank. They look up at us in the

window. They want entry. They want to be with us and live with us. But we never speak of them, never acknowledge their presence, never say their names or never recount their deeds to ourselves or each other. This is the only way to survive" (112). Attachment between a mother and a daughter, including kindness, care, love, carefulness and some limitations as well, ensures respect and will blossom. But a damaged relationship would have adverse psychological effects depression, attachment issues and hostility.

Adler believes that people yearn for belongingness. When someone feels connected and loved is at best mental status. Belongingness is important in families and absence of it may result in withdrawal, despair and depression. Munaweera has vividly portrayed the effects of presence and absence of belongingness in Ganga's life. Absence of belongingness with her relationship to her parents and later on her molestation makes her heart 'a creature hidden deep in its shell' and she says, "I turn my body into a castle, inviolate. If no one gets into my body, nothing dangerous can burst out of it, either. In this way safety is won"(128). Ganga enjoys the shower of belongingness when Daniel blossoms her body and heart with immense love and affection that makes her realise that "He needs me; I am special; I am chosen" (163) and "Just like that I know I will belong to this man for always. I, who have sailed these seas in storm and peril, who had felt ever wind-tossed, ocean-flung, have come home to dock in these safe and sunny harbours" (189). She does not want to remember her traumatic past and when Denial called her perfect and she says "loved the word *perfect*. Not a stain or a blemish anywhere on me" (188). The sense of belonging reaches to extreme when she becomes pregnant that made her realise "A child made by him and me would be born of love, even if accidently. And this accidental child... would love me unconditionally, and for this I yearn" (198). Thought of adoration, unconditional love, togetherness from these two people seems the goal in the life of Ganga. She thinks "here are the two souls who will love me without condition, without artifice. I know I will never again wander pathless and unsure of who or what I am. Instead I am this one's wife and this one's mother; I will be called by these sacred names: *wife, mother*. I will be fixed, stable, and held in place securely between them" (204). Ganga experiences the absence of belongingness in her marital life when suspected and feared Daniel announces to take Bodhi with him, leaving Ganga all alone, by saying her not to behave like her mother, who used to lock herself for hours leaving crying Ganga on the doorsteps. Shattered, broken down, disappointed, unwanted Ganga utters, "It feels as if someone has pierced my skin, pulled back the plunger on a syringe full of shame and shot it deep into me. When shame reaches and floods my heart, I know I have done what cannot be undone" (52). The traumatic past which she forgot in the company and belongingness of Daniel once again starts screaming under her skin "It wakes up and howls and wants to be seen, wants to show its broken face that is also mine. It asks for sympathy or perhaps for love. It screams that it is too was a child once and it was hurt. It asks why it cannot have these things: love, belonging, ease" (238). Ganga always remained in indecisive condition whether Daniel, her mother and her daughter share a sense of belongingness with her. Social connectedness has to be consciously developed to live in harmony which requires security, deep sense of belonging and embeddedness.

Adler opines "Society has an organic basis. The point of tangency between the individual and society may be found in the fact of man's bisexuality. Not in the isolation of man and woman" (*Understanding Human Nature* 33). Further he asserts, "Every child, dependent as he is on the help of the community, finds himself face to face with a world that gives and takes, that expects adaptation and satisfies life. His instincts are baffled in their fulfillment by obstacles whose conquest gives him pain" (*Understanding Human Nature* 34). Munaweera has raised a question on role of social aspects as well as role of family for healthy physical and psychological development of a child. Munaweera from the mouth of Ganga tries to examine the role of family and the utmost need of safety in a family by stating, "family is the place of safety. But sometimes this is the greatest lie; family is not sanctuary, it is not safety and succor. For some of us, it is the secret wound. Sooner or later we pay for the wounding of our ancestors. This was the truth for me and for my beautiful bright- faced child"(285). The molestation she endured in her childhood in her house by her father shook her belief on this pious relationship which resulted that later on she killed her own daughter. This is just because she had a dilemma that when a father from Asian culture where virginity "is the biggest deal. It's the matter of life and death. It's what mothers look for when they choose a bride for their sons."(158) Daniel, a foreigner who "grew up in America, where virginity is no big deal"(158) will do the same thing with Bodhi and she would suffer the same trauma with which Ganga is still suffering. Hans W. Roewald says,

The recognizing-caring activities of the primary caretakers crucially contribute to the development of the child's psychic life by the fact of their being of his present stage of organization, parental caring, knowing, understanding embedded in their interaction with the child, take place in the context and perspective of the child's overall requirements and future course of development, as perceived and misperceived by the parents. (*Psychoanalysis and the History of the Individual* 14)

Munaweera has explored lack of fulfillment of safety needs and protection by the family can provoke the victim to do an action which not only can harm him but also to his/her family as well as society.

Adler asserts, "The most important question which all women should ask their prospective husbands before marriage: 'What is your attitude towards masculine domination, particularly in family life?' is usually never answered" (*Understanding Human Nature* 126). Due to male dominance and excessive expectation from female for the fulfillment of her duties as wife or daughter or daughter- in- law in family she is always on stake for which Munaweera asserts, "We learn the rules of marriage. It is a closed system with its own weather, politics, and machinations. It is a loyalty constructed out of inside jokes, the sharing of fears, and predawn recounting of dreams. It is a small pot striving for the same patch of sun and in the process becoming entwined above and below ground" (193) and on the uncertainty of blissful married life she states,

Everybody knows that happiness in marriage is not expected. It is a possibility, of course, but it is not the reason one gets married. If it happens, one is lucky, but marriages are arranged for many reasons – financial, social, and as a calming agent on the hot tempers of young men and the possible waywardness of young girls. Happiness is hoped for but is never an expected consequence. (18)

but Ganga thinks that her uncertain mother, "will secure my future, my chastity and marriage to a good boy from a good family" (51). She herself shows lack of complete belief and trust on true marriage because she could share her past her husband. She remained in dilemma that if known "They will throw me out. I will be without family, without people" (71) because she takes in "this is my inheritance: silence and shame. A silence around the body with a better option to die than to break it. A shame so deep in the flesh" (252). She starts thinking that her husband and daughter will put her out of their mind and heart. She states, "There will be no memory of me. I will be erased. Our marriage would mean nothing. My motherhood would mean nothing. The way I loved them both would mean nothing" (253) and in dilemma says "Now I am that desolate thing, an abandoned woman left by her man, without her child" (252) and it results into a fatal action of killing her daughter to which she knows "It is perhaps the cruelest moment, but I can't do what was required" (237). This is all due to her irresolute attitude of not sharing her grief and problem to anyone. If she would have shared the past, her problems, her molestation and its outcome on her psyche to Daniel then perhaps she would have enjoyed a happy blissful life with her husband and her daughter. She would not have to endure the yoke of killing of her own daughter. She in dilemma says "I must save her. The way Amma never saved me. But I can save my little girl" (268) and tries to justify her action by saying "I am taking her away from people who could hurt her. Because you never know who could hurt a little girl. Sometimes it's the ones you trust most"(270). Munaweera has also portrayed Ganga's dilemma and indecisive mind because sometimes she thinks herself a completely cherished wife and mother but sometimes she feels herself completely detached from her husband and her daughter. In her abnormal state of mind knowingly she leaves her crying daughter alone for hours unless her cries become sobs and turn into silence. Coming to normalcy she says to herself, "I think I'll never do that again, never. I won't leave her despairing, unsure if I will ever return" (230). She is confused whether ever in her life she would be able to come out of her childhood abuse trauma which was covered her soul like a shroud. "I had longed for normalcy. I had wanted only these things – marriage, a shared place, the serenity of a long-lived love. But normalcy is a miracle, not granted to all who ask" (245). It illustrates that it becomes very hard for a female to show complete trust on any relationship if she endures physical assault.

Inferiority is a feeling which compels one to stay suppressed and secondary. In the novel Amma, an orphan, poor and seventeen years old, married Ganga's father, a wealthy orphan who supersede her monetary inferiority. But

Amma could not save her child from regular molestation. She even could not talk about it to her daughter. It was all due to her inferiority over male supremacy. Later on when the situation reached to the separation of the couple (Ganga and Daniel), Amma revealed the truth about molestation and said, "I was trying to protect you. So we never talked about it. I thought you couldn't remember. What happened with you when you were small. I should have stopped it"(267). Further she expresses her repentance "I am sorry. It's my fault... I tried to protect you. But I wasn't strong enough... No one would have believed me. No one would have taken us in" (267).This is a bitter evidence of the inferiority.

Truly, Child Sexual Abuse is a global matter and is not decreasing over time. Absence of faith, trust and attachment the relations decrease the blissfulness in life. Ganga, the prey of her mother's alienation and molestation distorted the proper psychological growth that resulted that she could not enjoy a happy married life. Hence, when the feeling of enthusiasm to contribute and connectedness are stronger, a feeling of sameness comes forward. Hence, several protective factors including social support, family support, parent-child relationships play a vital role for the whole development of a child.

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RESEARCH ARTICLE

Assess the Effectiveness of Fenugreek Drink on Menstrual Cramps among Adolescent Girls in Selected Rural Areas, Salem, Tamil Nadu

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ABSTRACT

A Quasi-experimental study, pre-test and post-test with control group interrupted time series design with quantitative approach was under taken to assess the effectiveness of fenugreek drink on menstrual cramps among adolescent girls in selected rural areas, Salem, Tamil nadu. 100 adolescent girls (50 Control &50 Experimental group) were selected by using purposive sampling technique and data were collected by Semi-structured Interview schedule, Observational checklist and Numerical rating pain scale. Finding revealed that highest percentage (65%) of the adolescent girls were in the age 15-17 years and 56.7% of them 18-19 years in experimental & control group, 63.3% and 56.7% of them had primary & high school, 63.3% & 40% of them belongs to Hindu & Muslim, 75.3% &63.3% of them belongs to nuclear family, 55.3% Rs.10001-15000 & 45% Rs.5000-10000 of them had family monthly income in experimental & control group.86.7% & 80% had non-vegetarian, 63.3% and 60% of them had sources of information from television, 66.7% &56.7% of them in 10-13 years had attained age of menarche, 66.7% & 63% had 28-35 days duration of menstrual cycle, 63% of them had 3-5- and 5-7-days duration of menstrual flow, 62% &56.7% of them had excess, 63.3% and 49.7% of them had day1 & 2 of menstruation period, 86.7% and 66.7% had pain during menstruation, 66.7% & 60% had pain after day1. 53.3% of them used 4-6 & 7-9 vaginal pads per day, 60% of them changed 4-6 hours once vaginal pads per day in experimental & control group. 80% and 68.7% of them had disturbances of daily activities. 53.3% of them had ability to manage daily activities extend, 67.7% and 63.3% of them had sleeping pattern disturbance, 53.3% and 50% of them had sleeping per day 8 hours in experimental & control group. Similar highest percentage of

the adolescent girls had moderate pain in control group 2nd& 3rdpost test. Hence, it shows that effectiveness of fenugreek on menstrual cramps among adolescent girls.

Keywords: fenugreek drink on menstrual, experimental study, days duration

INTRODUCTION

Adolescence period is the cross road period in life's development. This period of life is characterized by a steady progression of psychological and social adaptations, push the adolescent to learn and develop coping mechanisms that will be carried throughout the life. Puberty is the period at which maturation of the primary sexual changes take place (Swaminathan, 2017). Dysmenorrhea is a Greek word which literally means painful menstruation. They are abdominal and pelvic pains experienced before and during menstruation. Menstrual cramps may last for hours or up to three days. The cramps may be mild or severe pain it can interfere in regular activities or other work etc., (Lowdermilk.et.al, 2018).

Statement of the Problem

A study to assess the effectiveness of fenugreek drink on menstrual cramps among adolescent girls in selected rural areas, Salem, Tamil Nādu.

Objectives

- To assess the menstrual cramps before administering fenugreek drink among adolescent girls in experimental group and control group.
- To assess the menstrual cramps after administering fenugreek drink among adolescent girls in experimental group.
- To compare the effectiveness of fenugreek drink on menstrual cramps among adolescent girls with demographic variables in experimental group.
- To find out the association between pretest score of fenugreek drink on menstrual cramps among adolescent girls with demographic variables in experimental group and control group.
- To find out the association between post test score of fenugreek drink on menstrual cramps among adolescent girls with demographic variables in experimental group and control group.

METHODOLOGY

Research Design and Approach

A Quasi-experimental study, pre-test and post-test with control group interrupted time series design with quantitative approach

Study Setting

The study was conducted in Anaikuttapatti and Kalparapatti, Salem district.

Population

The study population comprised of the entire individual with the adolescent girlsliving in Anaikuttapatti and Kalparapatti, Salem.

Sampling

The study samples were adolescent girls living in Anaikuttapatti and Kalparapatti, Salem who fulfilled the inclusive criteria.

Sampling Technique
Purposive sampling technique was used as a sampling technique for the present study.

Sampling Size
100 adolescent girls living in Anaikuttapatti and Kalparapatti(50 control group and 50 experimental group) Salem.

Tool used
Semi-structured Interview schedule, Observational checklist and Numerical rating pain scale was used to collect the data regarding the effectiveness of fenugreek drink on menstrual cramps among adolescent girls.

RESULT AND DISCUSSION

100 adolescent girls were selected by purposive sampling technique and data were collected by using Semi-structured Interview schedule, Observational checklist and Numerical rating pain scale. The collected data was analysis by inferential statistics. Demographic characteristics reveals that highest percentage (65%) of the adolescent girls were in the age 15-17 years and 56.7% of them 18-19 years in experimental & control group, 63.3% and 56.7% of them had primary & high school, 63.3% & 40% of them belongs to Hindu & Muslim, 75.3% &63.3% of them belongs to nuclear family, 55.3% Rs.10001-15000 & 45% Rs.5000-10000 of them had family monthly income in experimental & control group. 86.7% & 80% had non-vegetarian, 63.3% and 60% of them had sources of information from television, 66.7% &56.7% of them in 10-13 years had attained age of menarche, 66.7% & 63% had 28-35 days duration of menstrual cycle, 63% of them had 3-5- and 5-7-days duration of menstrual flow, 62% &56.7% of them had excess, 63.3% and 49.7% of them had day1 & 2 of menstruation period, 86.7% and 66.7% had pain during menstruation, 66.7% & 60% had pain after day1. 53.3% of them used 4-6 & 7-9 vaginal pads per day, 60% of them changed 4-6 hours once vaginal pads per day in experimental & control group. 80% and 68.7% of them had disturbances of daily activities. 53.3% of them had ability to manage daily activities extend, 67.7% and 63.3% of them had sleeping pattern disturbance, 53.3% and 50% of them had sleeping per day 8 hours in experimental & control group. Similar highest percentage of the adolescent girls had moderate pain in control group 2nd& 3rdpost test. Hence, it shows that effectiveness of fenugreek on menstrual cramps among adolescent girls.

Percentage wise distribution to assess the effectiveness of fenugreek drink on menstrual cramps among adolescent girls in experimental group and control group reported that more or less similar highest percentage (72%& 70%) of the adolescent girls had moderate pain in control group 2nd& 3rdposttest and more or less similar lowest percentage (18% & 16%) of them had severe pain in experimental group 1stpost test and mild & moderate pain in control group 1stpost test& experimental group 3rdpost test.58%& 52% of them had mild & severe pain in experimental group 3rdposttest&pre test. Whereas, more or less similar percentage (48%, 46%, 44% & 40%) of them had mild & severe pain in experimental group 2ndposttest& control group pre test, moderate pain in experimental & control group 1stpost test and severe pain in control group 1stpost test. Further, more or less similar percentage (36%, 34%, 32%& 30%)of them had mild pain in experimental group1stpost-test, moderate pain in experimental group 2ndpost test, moderate pain in control group pre test and severe pain in control group 3rdpost test. More or less similar percentage (28%, 26%, 22% &20%) of them had severe pain in control group 2ndpost test, no pain in experimental group 3rdpost test& moderate pain in experimental group pre test, mild pain in experimental & control group pre test. Only 10% & 8% of them had severe pain & no pain in experimental group 2ndpost test. None of them had no pain in control group pre-test, 1st, 2nd& 3rd post-test, experimental group pre-test& 1stposttest. Hence, it can be interpreted that similar highest percentage of the adolescent girls had moderate pain in control group 2nd& 3rdpost-test, it shows that effectiveness of fenugreek drink on menstrual cramps among adolescent girls.

CONCLUSION

In the present study it can be concluded that effectiveness of fenugreek drink on menstrual cramps among adolescent girls. Hence, it can be interpreted that the investigator needs to conduct experimental study to assess the adolescent girls had average effectiveness of fenugreek drink on menstrual cramps.

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TableNo.1: Frequency and percentage wise distribution to assess the effectiveness of fenugreek on menstrual cramps among adolescent girls in experimental group and control group.

Level of Pain	Control Group								Experimental Group							
	Pre test		1st Post test		2nd Post test		3rd Post test		Pre test		1st Post test		2nd Post test		3rd Post test	
	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
No Pain	0	0	0	0	0	0	0	0	0	0	0	0	4	8	13	26
Mild Pain	10	20	8	16	0	0	0	0	11	22	18	36	24	48	29	58
Moderate Pain	16	32	22	44	36	72	35	70	13	26	23	46	17	34	8	16
Severe Pain	24	48	20	40	14	28	15	30	26	52	9	18	5	10	0	0
Overall	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**	**50**	**100**

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RESEARCH ARTICLE

Optimized Intuitionistic Fuzzy Controller Integrated Eagle Search Deep Neural Network for DDoS Attack Defence in SDN based Cloud

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ABSTRACT

In recent years, cloud computing combined with a software-defined network has achieved the goal of quick response to a changing environment based on the demand for and utilisation of cloud resources. The flexibility and versatility of SDN with Open Flow Controller and Switches, the message exchange and transmission of packets optimize the cloud network performance. However, security risks such as DDOS attacks, which deny services to genuine user's owing to high resource consumption and traffic interruption, are easily vulnerable even in SDN based cloud environment. Though, there are many machine learning-based approaches are available to handle the DDOS attacks, the presence of uncertainty in the behaviour of the source which initially pretends as a normal and later on it begins to flood forwarding packets to the open flow switch which results in high exhaustion of resources and traffic delays which stops service changing functionalities offered by NIDS, Firewall, internet, etc. To address the issue of suspicious sources' unpredictable behaviour in the cloud, a two-stage DDOS attack defencing mechanism that examines, validates, and screens incoming traffic before it is transmitted to the cloud's SDN Open Flow switches. In the first stage the uncertainty is handled by developing Intuitionistic Fuzzy Controller based History IP filtering technique (IFZ-HIPF). It represents parameters of SDN in terms of degree of pertinence and non-pertinence to classify the source as normal or illegal. In the second stage type of DDOS attack is analysed in depth by devising Eagle Search Deep Neural Network (ESDNN), which overcomes the over fitting problem of the existing Multilayer perceptron and SVM-SOM classifiers. The learning rate of the DNN is improved by adapting the intelligence of Eagle search algorithm instead of random assignment of weights in hidden layers of DNN. The simulation results proved that the performance of double filtering model IFZ-HIPF integrated with ESDNN

improves the DDOS attack detection rate and accuracy more prominently with reduced False Alarm rate by elevating resource depletion and packet loss ratio.

Keywords: Software Designed Network, Cloud, Uncertainty, DDOS attack, Intuitionistic Fuzzy Controller based History IP filtering, Eagle Search Deep Neural Network

INTRODUCTION

During the previous two decades, networks have been subjected to massive traffic requirements and criticism during document sharing, communication done internally, service provided and cost-based network resources usage [1]. Both corporate and individual requirements necessitate cloud computing. Cloud users notably enterprises, are increasingly storing sensitive and confidential data in the cloud because to the benefits of accessing high cloud resources [2]. Data in the cloud is secured by cloud service providers. Cloud computing will rely on hardware and software resources, which will typically be distributed across numerous Data Centres (DC) [3]. These cognitive resources are dynamically allocated, allowing service capacity to change in response to customer demand. The data centre faces the problem of flexibly as key issue in addressing the difficulty of planning and managing [4]. Systems with dynamically changing characteristics, workloads, performance, availability, and reliability goals. One of the network resources is Software Defined Networking (SDN) is a kind of network design which offer flexibility and reliability by unravels data plane and control plane [5]. By deploying SDN in cloud it gains the ability to react fast depending on the dynamic behaviour of the environment [6]. Another main issue in cloud is security threats which also rapidly detected by the SDN.

SDN centralize the intelligence of network as a single component by discriminating the data plane from the routing process to accomplish the flexibility in cloud computing as shown in the figure 1. The data plane also known as switches involves in process of forwarding network packets and Control plane also termed as controller handles the routing process which is the brain of the SDN [7]. To implement SDN in network equipment open flow is used for interaction among open flow switch and open flow controller. SDN is vulnerable to Distributed Denial of Service (DDoS) attacks because of its centralized management. There stricted resources of data plane switches, a significant amount of malware with spoofing addresses can be delivered to switches of open flow results in buffer overflow and flooding of flow table [17]. Moreover, switches are compelled to transfer a stream of packets in communications to the controller to request flow. This causes packet flooding on the controller, results in overloading. In general, DDoS attacks can cause network failure, making flow detection critical for SDN network security [18].When a system is subjected to a DDoS attack, the attackers typically transmit attack traffic to a particular target, leveraging a huge proportion of spoofed source IP addresses. A Network Intrusion Detection System (NIDS) is a victim of a Service Function Chain that delivers a public service, such as Web or FTP services, with in context of SDN. As a consequence, the volume of source IP addresses grows substantially in a quick period of time, as illustrated in Figure 2. Data captured in the flow table of an Open Flow switch is studied and various detection methods are submitted to detect DDoS assaults in SDN. When detecting DDoS assaults, SDN centrally regulates communication within the network. This results in a high processing demand, and the Open Flow controller's processing burden must be factored.

Problem Statement

Though SDN based cloud servicing is more flexible by effective management of resources and controlling network traffic based on the adverse environment, but it is more vulnerable to the DDOS attacks. A DDoS assault is a hostile attempt to degrade the resources of a device or network by feeding them continuous and massive traffic. The attacker's goal is to deplete bandwidth and deplete the cloud resources. The attacker starts a DDoS attempt by inserting a code known as botnet into the victim servers or network. Once the assault is launched, these codes are triggered, and a flood of data would be sent to the victim. Botnets provide toughness to the attack by keeping the attacker anonymous. There were already several research initiatives aimed at identifying and enhancing the security

of SDN which are very simple such as classification application and providing framework for security-based applications.

- While using MLP and SVM based classifier overfitting problem arises in detection of DDOS Attack in SDN
- Employing Binary classifiers will not provide further information about the type of attack detected.
- The traditional evolutionary modules cannot produce optimized response time in DDOS attack detection

Contribution

In this research work two different algorithms have been proposed to improve the DDOS attack deterrence in SDN based cloud.

- Deep learning with memetic based eagle strategy algorithm is developed for prominent classification of DDOS attack type by handling the existing problem of over fitting and binary classification.
- Intuitionistic Fuzzy based IP filtering scheme is applied as a defender to improve the attack detection rate by handling uncertainty in discriminating the IP addresses

Related Work

Cheng et al [8] designed a unique approach for predicting he attack behavior by collection the data form SDN controller. They constructed a switch flow tables with 6 tuples of features from the open flow switch flow tables. The extracted characteristics are fed as input to the SVM classifier to predict the presence of DDOS attack in SDN. Jankowski et al [9] offered a strategy for monitoring and detecting harmful activity in the SDN data plane utilizing SDN mechanism integrated with machine learning paradigms. The intrinsic processes of SDN technology create statistics and aspects of network traffic. To carry out tests and verify the concept, it was necessary to collect a workload test data of network. A simulated environment is constructed which allows SDN network traffic. In their research, effectiveness of Self-Organizing Maps and Learning Vector Quantization and its variants are used for DDOS detection.

Yang et al. [10] proposed a solution that combines IP entropy and flow information, resulting in a more precise detection effect. Whilst information entropy is useful and adaptable, it should be employed in concert with other approaches to control the threshold and different topics distribution of weight. Lin and Wang [11] offered an SDN-based DDoS assault diagnosis and coping strategy, however the method depends on Open Flow management tools and the Flow standard to perform outlier detection, rendering it difficult to establish and expand. In the defense against DDoS assaults, Abdullah et al [12] emphasize the importance of early detection and isolation of network traffic. This work suggests the deep neural network for identifying DDoS attacks on a subset of packets captured from data traffic. The DNN framework can respond efficiently and precisely albeit with low sample as it incorporates extraction of features and classification in its structure, along with layers that upgrade automatically as it is learned. Javed Ashraf in their work [13] they examine SDN in conjunction with the open flow protocol from the standpoint of DDoS attacks and intrusion detection, and proposes machine learning-based mitigation strategies.

Pahan et al [14] recommended combining SVM with SOM because they benefited from both algorithms: SVM provides excellent outputs in a short period, while SOM produces a reliable prediction relying on its neurons. The model then suggested a hybrid flow-based work mechanism in SDN that employs the recommended blend of SVMs-SOM to address DDoS assaults based on the network resource scarcity prevention. Hou et al [15] devised a deep learning and NetFlow features are used for identifying DDoS traffic. They employed real-time Net Flow samples to derive dynamic flow- and sequence characteristics. Random Forest is used to identify both normal and abnormal packets to prevent from DDOS attack and its variants. Hossesine and Azizi [16] aims to use two alternative methods based on machine learning models to detect RoQ assaults. They used several machine learning techniques to detect RoQ assaults, such as the Multi-layer Perceptron neural network with back propagation. Another approach is to integrate three different models to detect the RoQ attack more accurately.

Proposed Methodology: Intuitionistic Fuzzy Controller integrated Eagle Search Deep Neural Networkfor DDOS Attack defence in SDN based Cloud

The proposed research work aims to detect distributed denial of service attack in SDN based cloud by developing two stage filtering model. As the open flow controller and switches are highly vulnerable to DDOS due to its flooding nature. The heavy load of traffic consequence to server down or failure and the legitimate cloud users also be greatly affected because of denial of service. To overcome these issues in SDN with open flow switches and controller, the first phase is involved in designing an uncertainty-based history of IP database filtering model using Intuitionistic fuzzy inference technique is implied to normal and abnormal packets which reduce the false alarms and speed up the process of detection rate. In the second stage, the type of DDOS attack is classified by devising deep neural network with eagle strategy algorithm. This newly developed algorithm overcomes the problem of overfitting the class imbalance of attack types and improves the detection rate by integrating eagle search strategy to produce optimize attack detection rate and accuracy in detection of DDOS attacks in SDN. The simulation results proved the efficacy of the proposed IFZ-HIPF and EDNN for DDOS attack detection in SDN based service function chaining in Cloud. The overall flow of the proposed Intuitionistic fuzzy controller integrated with eagle searching deep neural network for DDOS attack detect in SDN is depicted in the figure 3.

Intuitionistic Fuzzy History based IP Filtering Model

Intuitionistic fuzzy expresses each real time factors in terms of membership and non-membership grade signified as μ and υrespectively [19]. Each data packet in DDOS attack detection is represented in the following format

$$F= \{z, \mu_F(z), \upsilon_F(z) | \ z \in Z\}$$

Both $\mu_F(z)$ and $\upsilon_F(z)$ values lies between 0 to 1 which satisfies the condition as denoted

$$0 \leq \mu F(z)+\upsilon_F (z) \leq 1$$

Considering the hesitation degree $\pi_F(z)$ is the most important component in intuitionistic fuzzy [20, 21], this constraint evidently expresses uncertainty that exists in DDOS attack detection in SDN is represented as shown

$$\pi_F(z)= 1-\mu_F(z)-\upsilon_F(z); \ 0 \leq \pi F(z) \leq 1$$

In this paper, to accomplish intuitionistic fuzzy based history-based IP filtering technique in SDN controller, the algorithm is applied in the application layer by partitioning the Intuitionistic fuzzy expert system into five different components as depicted in the figure. The input components receive the numerical crisp data of DDOS attack dataset, the second component converts crisp input values into intuitionistic values. The third component is intuitionistic fuzzy inference engine which inherits the acquaintance as well as logical verdict making. The final part is intuitionistic defuzzification which convert the output of the IFIS into crisp numerical value as shown in the figure 4.This proposed work discriminate normal sources from illegal legitimates, source parameters information is collected and maintained in the database. Let us assume that LS is the group of legitimate sources whose IP address is offered by the SDN cloud-based providers. Each source SIP_ihas a set of parameters to describe whether a source is a normal or illegal. The parameters of SIP_i are

- source active time SAT_i – detects how long the source has been connected to the server and also verifies that no traffic assault has occurred.
- Number of Incoming flows NIF_i of Source SIP_i - count the number of inbound flows to a cloud service
- Average number of packets per flow APF_i of source SIP_i –discriminate the normal source IP from the illegal counterpart
- Flag of SIP_i – status of the source SIP_i as normal (0) or attack (1) or indeterministic (-1)

With these parameters the intuitionistic fuzzification of these parameters are represented by membership and non-membership values as illustrated based on number of packets per flow To handle the uncertainty in differentiation of normal and abnormal source which attempts to access the SDN controller-based service chaining functionalities in cloud the intuitionistic fuzzy well clearly represent them as normal, abnormal or indeterministic which has to be provided additional observation. The following intuitionistic fuzzy rules are generated for distinguishing normal, abnormal or indeterministic source, by observing of the parameters of sourceSIP_i which communicates with SDN controller for accessing its services in cloud.

$RL_{NML}(SIP_i)$: If ($SAT_i$is low) & (NIF_i is Low) & (APF_i is Low) & (PRO_I is low) then (FLAG is Normal) [0.8]
$RL_{ATT}(SIP_i)$: If (SAT_i is High) & (NIF_i is High) & (APF_i is High) & (PRO_i is High) then (FLAG is Attack) [0.7]
$RL_{IND}(SIP_i)$: If (SAT_i is Medium) & (NIF_i is Medium) & (APF_i is Medium))& (PRO_i is medium) then (FLAG is indeterministic) [0.5]

The rule for Normal source is the parameters SAT_i,NIF_i, APF_i, PRO_ihas low range of values as its basic characteristics to be a legitimate source. If all the four parameters are high, then the source is in the attacking mode and its victims are serving changing functionality of the SDN such as website or internet or firewall. When the SAT_i,NIF_i, APF_I, PRO_i are holding medium values then it is very difficult to consider it as normal or abnormal so more attention is needed for the concern source in further observations.
The algorithm for Intuitionistic Fuzzy History based IP filtering for discriminating the normal and illegal sources in SDN based cloud is as follows
Input: SIP= (SIP_1,SIP_2,...SIP_n) set of sources, {SAT_i,NIF_i, APF_I, PRO_i} are the parameters of each SIP_i
Procedure

- Convert the input values of {SAT_i, NIF_i, APF_I, PRO_i} to Intuitionistic fuzzy Values

$$\mu_{SIP}(att) = \begin{cases} 0 & att \le a_1 \\ \frac{att-a_1}{a_2-a_1} - \epsilon \quad ; & a_1 < att \le a_2 \\ \frac{a_3-att}{a_3-a_2} - \epsilon; & a_2 < att \le a_3 \\ 1 \quad ; & att \ge a_3 \end{cases}$$

$$\upsilon_{SIP}(att) = \begin{cases} 1-\epsilon & att \le a_1 \\ 1 - (\frac{att-a_1}{a_2-a_1}) \quad ; & a_1 < att \le a_2 \\ 1 - (\frac{a_3-att}{a_3-a_2}); & a_2 < att \le a_3 \\ 1-\epsilon \quad ; & att \ge a_3 \end{cases}$$

- Generate the intuitionistic Fuzzy Rules by examining the knowledge base

If (SAT_i is low) & (NIF_i is Low) & (APF_i is Low) & (PRO_I is low) then (FLAG is Normal)
If (SAT_i is High) & (NIF_i is High) & (APF_i is High) & (PRO_I is High) then (FLAG is Attack)
If (SAT_i is Medium) & (NIF_i is Medium) & (APF_i is Medium))& (PRO_I is medium) then (FLAG is indeterministic)

- Perform De-Intuitionistic Fuzzification
  Output Flag = { 0, 1, -1}

In the second stage the sources are further analyzed in depth to discover their pattern of parameters using the deep neural network and the volume of huge traffic is well handled by overcoming the problem of overfitting due to the class imbalance of attack type forwarding packets. The DNN performance is fine tuned by eagle strategy algorithm to detect the type of attack and the forwarding packets are considered to be normal or abnormal by implementing the algorithm in OpenFlow switches. The detailed explanation of the eagle search enabled deep neural network is discussed in the next section.

Eagle search Deep Neural Network based DDOS Defense System

The attacks occur in SDN may ensue in data plane or control plane. If it is in data plane it is a standard attach which only affects very few hosts. While, attack take place in control plane it shuts down whole network of the cloud service chaining functionalities. As a result, the SDN must rely on flow installation rules to transmit new traffic flows, resulting in OpenFlow switch flow table failures. This problem compels the SDN controller to examine individual traffic and put in updated flow rules in switches, consuming both controller and switch resources.

Deep Neural Network

A kind of machine learning method which is developed similar to artificial neural network which is knowns as Deep learning model. It is represented as a nested deep hierarchy and these perceptions with more intellectual representation [19]. DNN consist of multiple layers in between the input and output layer The DNN works as a feed forward network, which transmits the input data to the output layer.

Virtual neurons are mapped by DNN and random numeric values which is knows as weights assigned to the links. The input data is multiplied with the weights and all the output of the intermediate node is summed as processed by the activation function [20]. The output value produces the single value which is compared with the target value of the dataset and its difference is treated as error rate. For mitigating DDOS attack detection in SDN based cloud services, DNN can work rapidly and accurately even with small set of datasets, because it has feature extraction and categorization operations in its construction itself and its layers are also updated. But major drawback in DNN is during the training phase, the parameters in the hidden layer are assigned in the random fashion using greedy descent algorithm [21]. Which works in the trail and error basis. It compares the expected output and actual output generated by the DNN and if there is more different then it has more error rate, with back propagation it reassigns the weight values until it reaches the specified iteration or there is no change in output. This extends the execution time of the algorithm which reflects in delayed response to the packet classification as normal or abnormal. This may result in traffic flooding due to the DDOS attack in open flow switch. This proposed work introduced the eagle search algorithm to improve the parameter assigned of DNN with its food searching nature. Eagle Strategy based optimization is divided into two parts and it is based on the foraging behaviour of the eagle [22]. To overcome both global and local optimization while seeking, they use Levy flights to fly and search for their prey. Levy walks are used for global searching, while differential evolution is used for local searching.

Algorithm ESDNN for DDOS attack detection in SDN controller-based Cloud

Input: CICDDoS2019 dataset S = (SIP_1,SIP_2,SIP_n) represents n number of source ip,
SIP_i = (SAT_i,NIF_i, APF_i.)parameters of Source IP
IFZ-HIPS Flag = (flg_1,flg_2,flg_3..flg_n) as output label of the corresponding sources. $i \in 1..n$
Procedure
Begin
Initialize the parameter B// selected sources, H // is the candidate set, $F=B \cup H$., m is the number of parameters in the source IP
Wt_h =0,
W_F :input weights , nominated with features in Wt_B
While $|B| \leq |m+1|$ do
Initialize candidate weight Wt_h= 0
Update the weight wt_h with Eagle Search Algorithm
Arbitrary initialization of parameters $W_{B=0}$
While (until termination condition met)
Levy flight based global exploration of weight searching
Compute the objective function and discover aauspicious solution
Concentrated local exploration everywhere a hopeful solution through discrepancy progress
If (best weight values detected) then
Update the current best

End
Update s = s+1
End
Multiple times drop out done to obtain average of GR_{Fj}

$$j = \max_{h \in H} \left\| GR_{F_j} \right\|_q$$

Perform B = B U F_j and H = H/F
End
Output: Classification = {Normal, DDOS Attack Type}
Where, B as selected set of sources, H as Candidate set ofsources and F = B∪H. The Input layer weights WF refers to input weights, Wt_B as the selected parameters weight, Wt_h denotes candidate weights, gradient of Wt_f isGR_f. GR_{fj} refers GR_f to choose one parameter such as jth one form H. Wt_{fj} refers to Wt_fwhich is arecently chosen input weights signifies the associated weights with j^{th}input in Wt_f. Then update B = B ∪ F_j and H = H/ F_j and continue the process for all the source set in the dataset.

RESULTS AND DISCUSSIONS

The performance of the proposed model Intuitionistic Fuzzy Controller integrated Eagle Search Deep Neural Network**(**IFZ-HIP+ESDNN) for DDOS attack detection in SDN based cloud is discussed in this section. The proposed model is deployed in python software. The CICDDoS2019 [25] dataset is used for DDOS attack detection on SDN OpenFlow controller-based cloud. The CICDDoS2019 dataset comprised of the source IP information flow with normal and DDOs attack packet information. TheCICDDoS2019 is a collection of benign and DDoS attacks that closely reflects real time data. The results of the network traffic study are also included. The proposed model IFZ-HIPF is compared with Standard HIPF and enhanced HIPF, the ESDNN performance is compared with two existing classification models known as MLP and SVM-SOM. The evaluation metrics used for performance analysis are abnormal source detection rate, packet loss ratio, traffic delay, CPU utilization, attack detection, accuracy and false alarm rate. The figure 7 illustrates the performance of the three different DDOS defence filtering mechanism namely HIPS, EHIP and proposed IFZ-HIPF implanted in the SDN controller of cloud environment. The highest rate of illegal source which affects the service of the SDN is detected by the proposed IFZ-HIPF because it intelligently handles the flow request with the intention of attacking the network by representing each parameters of source IP is the terms of degree of pertinence and non-pertinence. The source which acts as normal at its initial stage, becomes illegal and begins attacking the victims are more appropriately determined by the degree of hesitation in IFZ-HIPF and thus it detects highest number of abnormal sources in SDN controller. While other two existing filtering schemes focuses on the certainty attacks, the proposed IFZ-HIPF focuses on uncertain attacks also.

The traffic delay due to the DDOS attack in SDN based cloud on three different classification schemes is depicted in the figure 8. The result explores that the two-stage proposed model IFZ-HIPS integrated with ESDNN tackle the traffic delay very intellectually by handing the vagueness in discriminating the source as legitimate or illegal. By introducing the hesitation degree in history-based source IP filtering model, improves the classification of normal and abnormal sources. Once its rule is fired as abnormal then it immediately passes the message to the SDN controller to reschedule a new OpenFlow rule and implement it in the Open Switch at its preliminary stage itself. The eagle search deep neural network understands the vulnerability of the DDOS by depth investigation of database and updates the forwarding packet rules at right time with speedy process. Thus, the proposed ESDNN produced less traffic delay compared to the standard SVM-SOM ad MLP. The figure9 demonstrates the performance comparison of three different classification model for DDOS defence models based on their attack detection rate, accuracy and false alarm rate. The proposed ESDNN produced highest detection and accuracy rate of e 99.41% and 99.54% with least false alarm rate of 0.2%. The SVM-SOM classifiers detection rate is 98.47% and accuracy rate is 97.62% with false alarm rate is 6.40%. The MLP classifier detection rate is 89.2% and its accuracy is 90.1% and the FAR is 11.2%. The ESDNN is better than MLP and SVM-SOM because it handles over fitting problem and class

imbalance in DDOS attack type more prominently with the intelligence of eagle searching which improves the learning rate of the DNN. The figure 10 explores that performance of three different classification algorithm for packet loss rate during DDOS attack in SDN based cloud. The OpenFlow switches are affected by overflooding and the servers are down due to the overloading of inflow packets which results in denial of service to the legitimate users in accessing service chaining facility offered by the cloud service providers. The Deep neural network understands the depth pattern of the input information about the sources with the gained label of intuitionistic fuzzy history-based IP filtering which handle the indeterministic behaviour of the attacking sources very accurately and reduce the packet loss rate which is considerably very less, compared to the SVM-SOM and MLP. The existing two classification models, when they receive huge volume of packet inflow the problem of over fitting arises and affects their performance and vagueness nature of the source with attacking mode is not appropriately detected by them. Thus, the proposed EDNN with the ability of eagle searching capability improves the weight parameters of the DNN in training and testing phase of the model to achieve least packet loss rate by detecting the abnormal source at right time and reframing a new flow rule to the OpenFlow switch. The performance analysis of CPU utilization of the classification models based on the traffic load is shown in the figure 11. From the obtained result it is observed that CPU utilized by the proposed ESDNN is very less compared to the MLP and SVM-SOM. The reason is the ESDNN detects the DDOS attack in SDN rapidly and the process of classifying more number of sources requesting services of the cloud is controlled by speeding up the rule generation and improving the learning rate of ESDNN with the limited iteration. The uncertainty issue is handled at the initial stage itself by IFZ-HIPF which fires the appropriate rules to categorize the source as normal, abnormal or indeterministic for its vague behaviour. The eagle searching model reduces the iteration of training phase in back propagation to adjust the weight parameters of DNN by assigning best weight values instead of random assignment.

CONCLUSION

The ultimate objective of this paper, is to design and deploy an optimized DDOS defense mechanism in SDN based cloud in a rapid manner. The standard existing algorithms SVM-SOM and MLP lacks its quick response to decide about the legacy of sources when more volume of forward packets is flooded in OpenFlow controller of SDN. To overcome this issue the double filtering process is adapted in this work by designing a uncertainty theory based Intuitionistic fuzzy controller which infers the characteristic of each sources in need of service with the help of hesitation degree. The History based IP filtering done by Intuitionistic Fuzzy controller greatly improves the detection rate of normal source and attacking sources. Further, the pattern of the attacking sources even it has less sample of dataset is intelligently handled by the proposed Eagle search Deep Neural Network, which speeds the detection process and triggers the immediate action against the forwarding floods by passing the message to the SDN controller. By enhancing the learning rate of DNN with the intelligence of Eagle food searching behavior the best values are assigned to the parameters. The simulation results proved that the proposed IFZ-HIPF integrated with ESDNN produced highest rate of DDOS attack with reduced false alarm rate.

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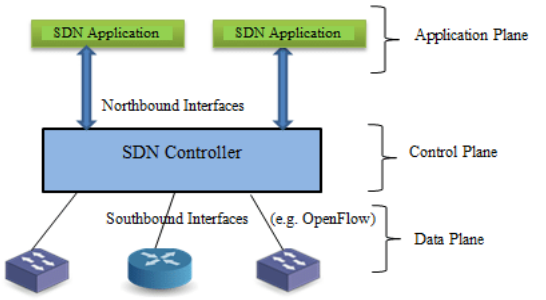

Figure 1: Simplified view of Software Designed Network

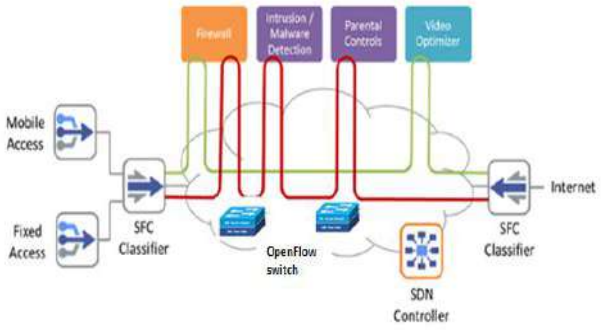

Figure 2: SDN architecture with SFC classifier

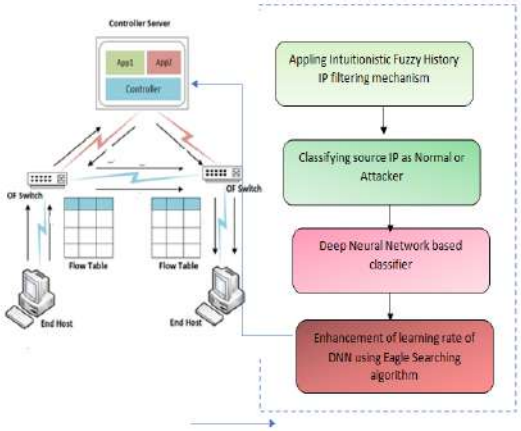

Figure 3: Overall Flow of the proposed model Intuitionistic fuzzy History based IP filtering with Eagle Search Deep Neural Network for DDOS defence in SDN enabled Cloud

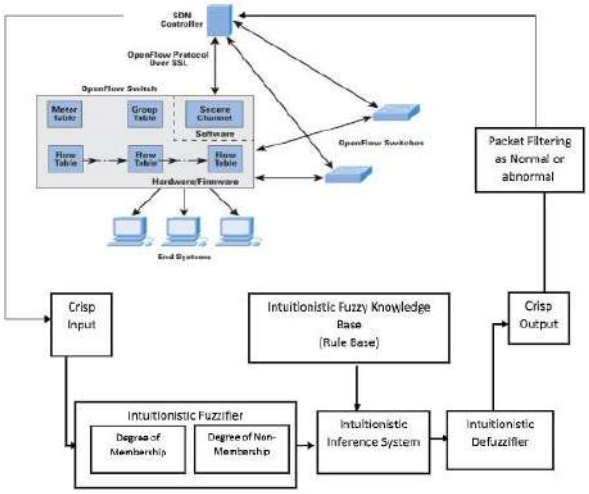

Figure 4: Intuitionistic Fuzzy Inference based IP filtering for SDN DDOS Attack Detection

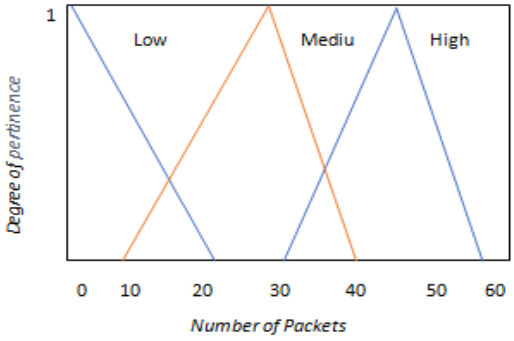

Figure 5: Degree of Membership of number of packets

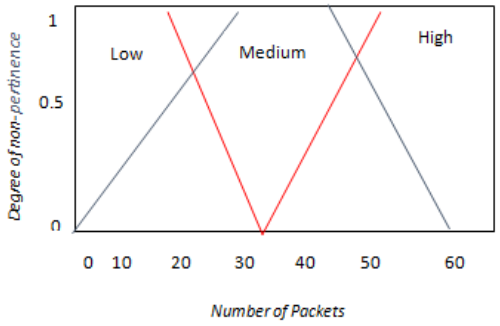

Figure 6: Degree of non-membership of number of packets

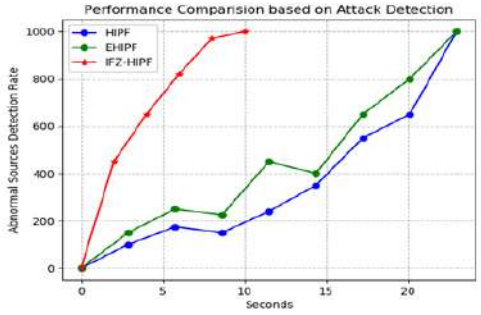

Figure 7: Comparison based on Abnormal sources Detection rate

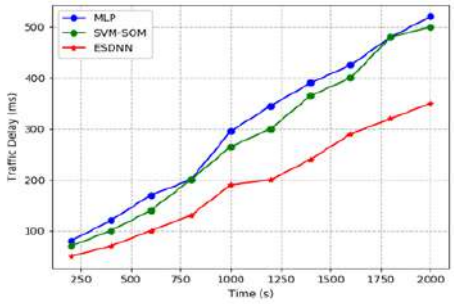

Figure 8 :Comparison based on Traffic Delay

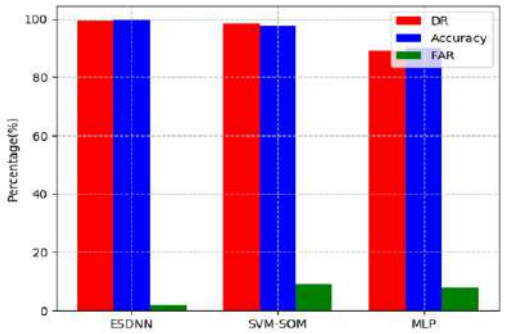

Figure 9 :Comparison based on Detection Rate, Accuracy and FAR

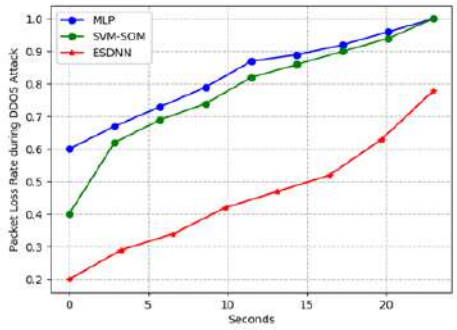

Figure 10 :Comparison based on packet loss rate during DDOs attack

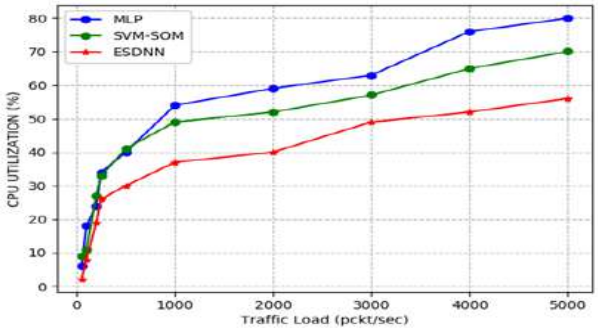

Figure 11: Comparison based on CPU utilization

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RESEARCH ARTICLE

Unveiling the Impact of Work Environment on Workplace Happiness - A Study on College Teachers of Cooch Behar District of West Bengal

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ABSTRACT

Happiness is the essence of every human being. Happy state of mind can perform any work well. Working professionals spend the major time of their life at workplace. Workplace happiness is essential to keep them mentally and physically fit. It is happiness of employees which inevitably brings miracle at workplace. Unhappy state of mind makes dull and dissatisfied face. An unhappy mind cannot give full effort from core of the heart which in turn makes them non-productive and cost effective for the organization. Cost effectiveness leads them to follow exit road. But exit strategy is an ailment, not the solution. Workplace environment plays a pivotal role in bringing employee happiness. While the work environment is favorable, employees sense a feeling of happiness at workplace and vice versa. Teachers play a major role in shaping the society and nation. Teachers' happiness is crucial. While teachers are happy, by products of them i.e. students can get the positive vibes. At college level, teachers are assigned with multidimensional duties and responsibilities within the campus and also beyond the campus. Those multidimensional duties and responsibilities along with the work environment (such as relationship with coworkers, student's support) puts them under huge mental pressure. If work environment favors them, they become happy and perform their duties and responsibilities well. In the current study, a snap shot of teachers of government and government aided colleges has been taken into consideration to find out how the environment affects the state of happiness among them.

Keywords: Work environment, Work place happiness, College level, Teachers.

INTRODUCTION

How to keep employees happiness at workplace is a matter of concern these days. An initiative has been found on the nib of the researchers and psychologists in this concern. They down their pen on why, where and how of happiness. Working professionals discern that it is not monetary benefits which keep them happy at workplace. To become happy at workplace an individual urge to be in such an environment which favors them. Work environment is the genesis of workplace happiness. Favorable work environment is the dominant spring of workplace happiness. Work environment is inescapable and multidimensional. There are numerous components of work environment which keep happiness among employees. It is due to the inseparability of employee and work environment, the work environment plays a pivotal role to keep the employees' state of mind happy or unhappy. If environment is unfavorable, employees sense the state of happiness on the contrary, unhappiness is inevitable. Teachers are not the mere icon of nation builder. In fact the nation rests upon teachers. Teachers' happiness is crucial. The happiness of teacher rests upon the work environment which surrounds them. In college level, there are many work environment related factors which affects workplace happiness of college teachers. There is cogent reason why people in one environment have positive attitude towards workplace and work while others in other work environment have reluctant and negative attitude towards it. Toxic work environment is the dominant spring of negativity towards work. Work environment inside the college campus affects the mental state of college teachers. Political activities inside the campus, conflict among teachers and administration, deteriorating student teacher bonding, no specific time boundary of work are some of the cogent environmental components of workplace unhappiness among college teachers. In the current study, three major work environment components like working hour, relationship with coworker and student teacher relationship has been taken into consideration.

Objectives of the study:

The current study has the following objectives:

1. To find out the state of happiness among college teachers.
2. To find out how college work environment affects the workplace happiness of college teachers.

Literature review

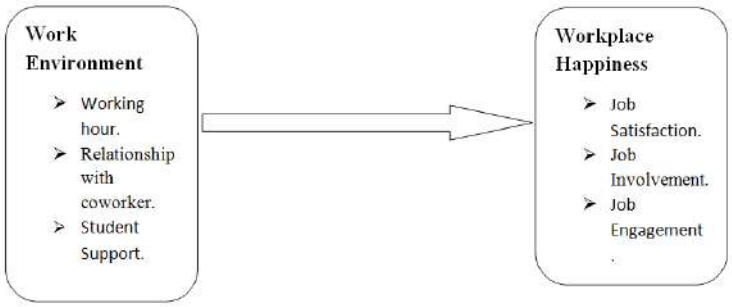
A supportive work environment permits an employee to try new things and don't get scared of if failure is the consequence (Kahn, 1990). According to Fisher & Fraser (1990), there are eight dimensions of a school-level environment. Some of those dimensions are 1) student support (2) affiliation (3) professional interest (4) staff freedom (5) participatory decision making and so forth. According to Spector (1997) ignoring the working environment turns an adverse effect on employee performance. He further added that employee safety, job security, cordial relationship with co-workers, recognition for good performance, motivation for good performance, and participation in the decision-making are favorable work environment factors. according to him if the workplace considers employees valuable and important, employee commitment and sense of ownership arose.A positive work environment positively affects numerous working conditions like employee safety, stable relations among peers, employees' participation in decision-making processes, and so forth (Spector, 1997). The declining status of professionals is due to inadequate working conditions (Ingersoll, 2001). Various workplace aspects affect employee engagement (Harter et al., 2001; Miles,2001; Holbeche & Springett , 2003; May et al., 2004 and Rich et al., 2010).

Different aspects of workplace affect employee engagement (Miles, 2001; Harter et al., 2002; Holbeche & Springett 2003; May et al., 2004; Rich et al., 2010). According to international research evidence, the reason for inclining turnover of educational institutions is a dissatisfied working environment with teachers' diminishing prestige dissatisfaction (Ingersoll & Smith, 2004; Borman & Dowling, 2008; TemaNord, 2010). A study by Rhodes et.al (2004) on 368 Primary and Secondary Education teachers in England concluded that work satisfaction is positively related to relationships with colleagues, work towards common goals achievements, exchanging experiences with colleagues, and the climate of success at school. A healthier working environment results in more employee engagement (Lockwood, 2005).According to many research papers, there is a strong and significant positive

relationship found between the working environment and the intrinsic aspect of job satisfaction (Gazioglu and Tanselb, 2006; Skalli, et.al. 2008). Barry (2007) stated that the work environment is an essential part of the work itself. A good many factors of working environment like wages, working hours, autonomy to employees, organizational structure, and communication between employees and management affect job satisfaction (Lane et.al., 2010). Job satisfaction is enhanced by salary, working hours, employees' autonomy, or¬ganizational structure (Lane et al., 2010). Working environmental factors like employee wages, working hours, the autonomy is given to employees, smooth communication between employees & management affect employee job satisfaction (Anne et al., 2010). Job-related stress, declined occupational safety and health, stress causes lower workplace happiness (Kiriago & Bwisa, 2013). A significant relationship between work environment and employee engagement has been observed by Anitha (2014). Anitha (2014), found a significant and positive relationship between work environment and employee engagement. Some important favorable factors of job satisfaction are work content and public contact and major unfavorable factors are lack of autonomy at the workplace and difficulty in transferability of qualifications (Kaiser, 2014). A study by the American Society of Interior Designers [ASID] (2015), found that job satisfaction is affected by the physical workplace. The workplace condition, the psychosocial atmosphere at the workplace helps in improving the performance of employees as well as the organization (Nyamwamu et al,. 2015). The key determinant of employee engagement is a meaningful work environment (Anitha, 2014;Popli & Rizvi, 2016).

Employees work more effectively in a conducive work environment (Khuong & Le, 2014). There is a strongly positive and significant relationship between collegial relationships and teacher commitment (Jo, 2014). There is strongly positive and significant relationship that persists among school climate and teachers' commitment (Raman et at, 2015). They further added that a higher level of school climate leads to a higher level of teachers' commitment. The collegial relationship can be improved by sharing knowledge and being cooperative with teachers which in turn enhances organizational commitment (Raman et at, 2015). Work environment is one of the critical factors which affects employee productivity and job satisfaction (Wilson 2015). The working environment at the school level is related to organizational commitment (Tran & Le, 2015). Employees' sense of well-being and commitment toward an organization is influenced by the work environment (Hanaysha, 2016). Data collected from 35 countries worldwide from the Teaching and Learning International Survey (TALIS) 2013 reveals that internationally teachers' job satisfaction rests upon student discipline and teacher cooperation (Sims, 2017, 2018). While teachers are in a comfortable and friendly environment, the school gets greater potential (Khadir et al.,2018). A good work environment improves employee loyalty, level of commitment, efficiency & effectiveness productivity (Khadir et al.,2018).

Independent Variable Dependent Variable



Hypothesis

H1 a: Working hour leads to job satisfaction.
H1b: Working hour leads to job involvement.
H1c: Working hour leads to job engagement.
H2a: Relationship with coworker leads to job satisfaction.

H2b: Relationship with coworker leads to job involvement.
H2c: Relationship with coworker leads to job engagement.
H3a: Student support leads to job satisfaction.
H3b: Student support leads to job involvement.
H3c: Student support leads to job engagement.

RESEARCH METHODOLOGY

Study Area: The entire study area has been conducted on the government and government affiliated college teachers of Cooch Behar District of West Bengal.

Sample Size: Sample size is 215.
Sampling Technique: Convenient sampling.
Data Collection Technique: Structured questionnaire method.
Research Design: Descriptive research design.
Statistical tool used: Pearson Correlation.
Statistical software used: SPSS 20.

Data collection: Data has been collected from the teachers of Government and Government Aided Colleges situated at Cooch Behar District of West Bengal. Proposed sampling technique was random sampling. But while actually data collected took place, the sampling technique was converted into convenient sampling because of the Covid 19 situation which hindered the collection of data. Totally 300 questionnaires were distributed of which 215 filled up questionnaires were returned by the respondents.

Data Analysis
A Pearson product-moment correlation was conducted to examine the relationship between different dimensions of work environment (working hour, relationship with coworker and student support) and different dimensions of work place happiness (job engagement, job involvement and job satisfaction). In case of correlation between working hour and job engagement, r value is .265 and the p value is .000(<0.01) which indicates that the correlation is significantly positive. The r value of relationship with coworker and job engagement is .251 where the p value is .000 (<0.001) which indicates that the correlation is significantly positive. Student support is significantly and positively related with job engagement (correlation value is .227 and p value is .001(<0.01). Working hour and job involvement {r = .264, p value = 0.000 (<0.01)}, relationship with coworker and job involvement { r = .259, p value = 0.000 (<0.01)} and student support and job involvement { r = .564, p value = 0.000 (<0.01)} are significantly and positively correlated. Working hour { r = .377, p value = 0.000 (<0.01)}, relationship with coworker { r = .325, p value = 0.000 (<0.01)} and student support { r = .363, p value = 0.000 (<0.01)} is significantly and positively correlated with job satisfaction.

FINDINGS

From the above mentioned data analysis, it is translucent that all the dimensions of work environment are positively and significantly correlated to work place happiness which also ensures that all the above mentioned hypothesis are being accepted. Thus the impact of work environment on workplace happiness of college teachers of Cooch Behar district of West Bengal is significantly positive which indicates that work environment plays a pivotal role in bringing happiness among college teachers.

CONCLUSION

Though happiness is our indigenous nature, but for working professionals, work environment is a strong determinant of happiness. College teachers' job is multidimensional. Their duties and responsibilities are not akin to other jobs. Teaching is a combination of all jobs. The work environment has its significant impact on their happiness. The current study reveals that work environment of colleges teacher has its significant impact on work place happiness of college teachers of Cooch Behar district of West Bengal.

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Table 1. Descriptive statistics and correlation coefficients for study variables.

Variable	M	SD	1	2	3	4	5
1.Working Hour	2.39	0.56					
2.Relationship with Coworker	2.04	0.665	.320**				
3. Student Support	1.93	0.727	.155*	.383**			
4.Job Engagement	2.03	0.607	.265**	.251**	.227**		
5.Job Involvement	2.13	0.647	.264**	.259**	.564**	.442**	
6.Job Satisfaction	2.2	0.633	.377**	.325**	.363**	.312**	.533**

**p < 0.01 level (2-tailed); *p < 0.05 level (2-tailed); N=215

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RESEARCH ARTICLE

Pharmaceutical Excipients: Regulatory Environment

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ABSTRACT

The expected capacity of an excipient is to go about as the transporter (vehicle or premise) or as a part of the transporter of the dynamic substance(s) and, in this manner, to add to item ascribes, for example, soundness, biopharmaceutical profile, appearance and patient adequacy and to the simplicity with which the item Almost all medication measurements structures incorporate some sort of excipient to ensure the dose, steadiness, and bioavailability. As of now, roughly 1000 excipients of in excess of 40 useful classes are utilized in advertised drug items. Customarily, excipients were frequently basically basic, organically latent, and of normal root. In contrast to API's, there is no conventional guideline for excipient make; despite this, the International Pharmaceutical Excipients Council (IPEC) has developed a comprehensive rule to assist manufacturers in excipient control. These rules, along with guidelines such as 21CFR 820, can be used to justify a provider's overall methodology for providing high-quality, well-documented basic crude materials to the pharmaceutical and biopharmaceutical industries.

Keywords: Excipients, Industry, API, IPEC, 21CFR 820

INTRODUCTION

Depending of the substance or manner of administration, pharmaceutical dosage forms must fulfil a number of basic features in order to assure and maintain efficacy, safety, and quality. In serial manufacture, only few active compounds can achieve these requirements. Other ingredients must be added to the formula to make up for the

identified shortfall. As a result, auxiliary chemicals (excipients) and active compounds are included in the formulation of all dosage forms. As a result, the function, throughout the design, development, and manufacturing of pharmacological dosage forms, the behaviour patterns and composition of excipients should be thoroughly studied. The term "excipient" derived from the Latin word "excipere," which means "take, collect, bring together, remove." Excipients have such effect on the dosage form's "weight, consistency, and volume" in order for the active drug to be administered to the patient effectively. In 1957, excipients, the medium through which drugs are distributed, were deemed unsatisfactory. To put it another way, they're inert auxiliary compounds that aid the active substance (s).

The excipient was defined in 1974 as "inert substances added in different quantities to the prescription in order to bring the medicine to the right consistency or shape." The work of "adjuvant in the transport and release of the active ingredient" As pharmaceutical research has progressed, more and more to this simple excipient duty has been added. Excipients are any substance that has been proven to be safe and is used in a release system to aid production processes, preserve, endorse, or improve stability, bio-availability, and patient compliance, aid in product identification/segregation, or improve the overall safety and effectiveness of the drug delivery system during storage or use [1].

Many advanced dosage forms are complex systems with various aspects in addition to These chemicals are frequently administered in addition to the active pharmaceutical ingredient (API) in the following order:-
Maintain, preserve, or improve formulation strength: - Excipients are added to normalise the active pharmaceutical ingredient, which aids in maintaining the product's stability and ensures that the API retains its strenght for a significant period of time.

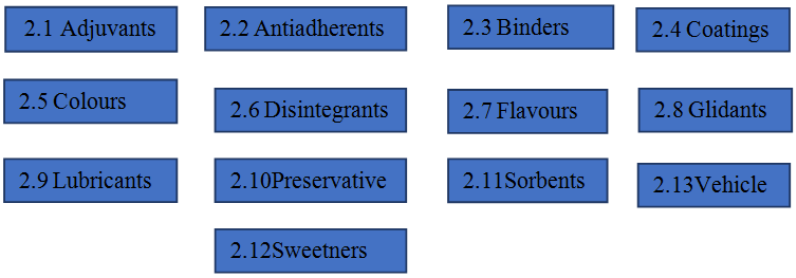
Help improve bioavailability of active drug: -Excipients, for example, typically aid in increasing the bioavailability of the active medicinal ingredient. In many circumstances, an active chemical (such as aspirin) is not easily absorbed by the human body. In some circumstances, the active component is dissolved in or blended with an excipient, which might function as a solvent or help in the medicine's absorption in the human body [2]. Just a few examples include accurate and full dosing, improving patient compliance, regulating drug release from dosage forms, targeting medicines, providing a protective impact, and optimising manufacturing procedures. Excipients are derived from a number of different sources and, for the most part, have no commercial designations. Excipients can be obtained from a variety of sources, which is listed below.

1. Animal sources: lactose, stearic acid,gelatin, honey, bees wax, musk, lanolin, and so on.
2. Vegetable sources: Include starch, turmeric, guar gum, peppermint, arginates, and acacia, among others.
3. Synthetic obtained: - Boric acid, Saccharin,Polysorbates, , Lactic acid, Polyethylene Povidone glycols, and other synthetic products were obtained [1].

Fillers, binders, dispersants, lubricants, flow correctors, sweeteners, colourants, and other substances are used in the pharmaceutical industry for a variety of purposes. Excipients come in a wide range of chemical forms, from basic molecules like water to complex, semisynthetic, or synthetic blends. Excipients can be divided into three categories from this perspective. These sub-sections are as follows:

1. Excipients that had been approved for use in pharmaceutical products for a long time, usually from the nutraceutical industry (generally recognized as safe).
2. Those obtained by modifying the structure of already excipients approved by the FDA or those used in the food/cosmetics business (Essentially New Excipients),
3. Those that have never been used in the pharmaceutical industry previously. Active research is being conducted, notably on excipients in the third category, to answer the rising interest in controlled release and the needs of today's high-efficiency tablet machines, and many breakthroughs have been realised. [3]

TYPES OF EXCEIPENTS [4]



REGULATORY REQUIREMENTS

Where a monograph exists, the excipient is relied upon to consent to the monograph just as different prerequisites in the pharmacopeia General Notices and appropriate required General Chapters. In numerous locales, including the US, EU, UK and Japan, pharmacopeia consistence likewise incorporates production to suitable GMP norms, which is noted in either the General Notices or different guidelines. Not all excipients are the subject of pharmacopeial monographs. This will fluctuate for various nations or worldwide areas. What's more, for certain materials utilized as excipients, other lawful prerequisites identified with organization may likewise apply.

For all excipients the accompanying focuses should be surveyed:

- Pharmacopeial necessities, when suitable
- Country/provincial prerequisites
- Manufacturer prerequisites (grade separation, GMP, and so forth)
- User prerequisites (remembered for administrative filings)

The International Pharmaceutical Excipients Council Federation (IPEC Federation) states that the altered IPEC Excipient Information Package User Guide and Templates (EIP) are now available. The guide was initially distributed in 2005, and along these lines amended in 2009 and 2013 and is broadly utilized around the world. The essential objective of the EIP control is to encourage the excipient provider's sharing of data with the client in a normalized route as opposed to finishing singular polls and reviews. The EIP comprises of 3 distinct archives covering quality frameworks, item administrative and production network and security:

- Site quality overview
- Product regulatory review
- Supply Chain and Security Overview

Formats for every one of these archives are accessible independently. The guide will be accessible, at first only to IPEC individuals for a three-month time span, on the IPEC Federation and public/territorial individuals' sites. From that point, the guide will be unveiled accessible to the general [5].

Current regulatory status of new excipients

Despite the fact that excipient innovators are capable of adapting Newer excipients are not making their way into pharmaceutical goods under the existing paradigm, even if companies are open to new methods and prepared to invest in research and safety evaluation. Due to the large investments required for pharmaceutical research, pharmaceutical businesses are naturally risk averse. Despite these obstacles, the FDA's increasing Inactive Ingredient Database (IID) indicates that innovative excipients are in high demand. Excipients used in permitted pharmaceutical items are included in the IID, together with their route of administration and maximum dose (i.e., maximum potency per dosage unit). A regulatory strategy that incorporates a thorough and predictable review of excipient safety and efficacy might result in a flood of new possibilities for drug formulators. Novel excipients are not reviewed independently in current medication approval procedures; they are only considered in the context of the initial drug application incorporating the excipient. Because a new excipient is a one-of-a-kind molecule, there is no regulatory approval process. Although the International Conference on Harmonization (ICH) does not have global excipient

safety evaluation guidelines, FDA excipient safety evaluation guidance cites several ICH safety-testing guidelines as reference materials for the conduct of safety tests (e.g., ICH S1A, S2B, S3A, S5A, S7A, and M3).[6]

INVENTION OF THE INTERNATIONAL PHARMACEUTICAL EXCIPIENTS COUNCIL

The goal of IPEC is to promote the harmonisation of various standards for the manufacture and use of pharmaceutical excipients, as well as the development of better consumer safety in the manufacture and use of pharmaceutical excipients and the introduction of innovative pharmaceutical excipients. There is currently no worldwide harmonisation of the various national excipient regulatory registration systems. The lack of regulatory provisions will be identified by IPEC.

The International Pharmaceutical Excipients Council (IPEC) is a global industry association of pharmaceutical, chemical, and food processing firms that develop, manufacture, trade, and use pharmaceutical excipients. IPEC is comprised of three regional organisations: the US, Europe, and Japan. IPEC has the same purpose in terms of International Harmonization of Excipient Standards. The major aim of the IPEC is to introduce innovative excipients to the market and to develop inviolability evaluation criteria. The IPEC Safety Committee (SCIPEC) is made up of certified scientists who develop safety testing for excipients. The guidelines focus on the excipient's chemical and physical properties,exposure, condition (including dosage, dose duration, frequency, method, and user population), and the presence or absence of pharmacological action are all factors to consider.

The IPEC set guidelines for evaluating new excipients' safety, as well as a guide for good manufacturing practise for bulk pharmaceutical excipients. The recommendations give enough information to determine the safe conditions of use for novel excipients. Technical advice papers for drug products containing novel ingredients have been approved by the ICH. Excipients are regulated by strict standards, or monographs, published by three major Pharmacopoeias in the United States, Japan, and Europe. Pharmacopoeial Harmonization also aids in the avoidance of unduly lengthy regulatory approval processes while ensuring product quality, safety, and efficacy. According to the Pharmacopoeial Discussion Group (PDG), which was created in 1989, harmonisation can be done retrospectively for current monographs or chapters or proactively for future monographs. Currently, 25 of the 35 main chapters have been harmonised, as have 39 of the 62 excipient monographs.[7].

The IPEC (later IPEC-Americas) is made in the U.S. with four fundamental objectives:

- Harmonisation of compendial norms for excipients;
- Harmonisation and proper GMP rule to guarantee excipient quality and consistency since no formal administrative survey and endorsement measure exists for drug excipients;
- Harmonisation and fitting security assessment rule for new excipients;
- Effective correspondence and collaboration with makers, excipient clients, pharmacopeia authorities and government controllers.

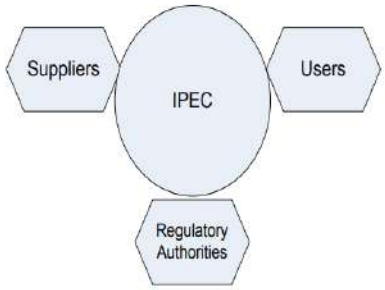
The Inactive Ingredient Database gives data on latent fixings present in FDA-endorsed drug items. This data can be utilized by industry as a guide in creating drug items. For new medication advancement purposes, when a dormant fixing has showed up in an endorsed drug item for a specific course of organization, the latent fixing isn't viewed as new and may require a less broad audit whenever it is remembered for another medication item. For instance, if a specific dormant fixing has been affirmed in a specific measurements structure at a specific strength, a support could think of it as safe for use likewise for a comparable kind of item.[8]

Excipient responses have come about because of the utilization of unmistakably poisonous substances (for example diethylene glycol), the utilization of certain excipients in a vulnerable gathering (for example low birthweight children, patients with enormous surface zone consumes, patients with a background marked by asthma or contact dermatitis), the modification of an excipient blend bringing about changed bioavailability (for example phenytoin), and the conscious or accidental extradural organization of saved drugs planned for intravenous use. Accidental

excipient overdose has likewise happened when abnormally enormous portions of a medication containing an additive were utilized [in morphine, ethanol in glyceryl trinitrate (nitroglycerin)]. Most excipient issues are preventable with information on the at present accessible plan. Government drug administrative organizations have to a great extent forestalled presentation of another poisonous excipient; nonetheless, the new utilization of recently affirmed (however not enough examined) excipients keeps on bringing about sad misfortunes (for example the E-ferol occurrence). Populaces in danger should be observed cautiously. Low birthweight babies (under 100g) have a very much shown prejudice to numerous excipients, especially during the initial fourteen days of life. Examination should be coordinated toward advancement of non-safeguarded drugs and more secure diluents for this populace. Medications and excipients which have recently been exhibited to be more secure in different populaces (for example doxa pram) should be carefully concentrated in this age bunch before broad use is suggested. Asthmatic patients contain another populace that is as often as possible touchy to excipient poisonousness. Now and again, as in sulphation specialists, who are omnipresent in nourishments just as in meds, all out evasion may not be conceivable and prophylactic treatment might be valuable. Dormant fixings are plainly not reliably inactive in their organic movement and subsequently ought not to be recorded in that capacity.

A more valuable and succinct term is excipient. It is strongly suggested that all drug makers list all their excipients and make this accessible to experts and medication data focuses. Then again, or also, the bundle supplement should list these excipients as per great assembling systems. This divulgence will assist with deciding the overall recurrence and extent of issues (bioequivalence, harmfulness, and so forth) that excipients may have in the populace, just as empowering defenseless patients to dodge coincidental presentation [9]. The IPEC Federation represents the five existing regional International Pharmaceutical Excipient Councils (IPECs) - IPEC-Americas, IPEC Europe, IPEC Japan, IPEC China, and IPEC India - and speaks with a unified voice to advance the best utilisation of excipients in medications as a method of improving patient treatment and wellbeing. IPEC has three major partner gatherings:

1. Excipient makers and wholesalers who, in this record are viewed as providers
2. Drug producers, (alluded to as clients)
3. Administrative Authorities who manage medications



Guidelines available in IPEC are

1. IPEC GMP Certification Scheme and CB Qualification Guide
2. IPEC Quality-by-Design Guide
3. Composition guide of IPEC excipient
4. Package information of IPEC excipient
5. IPEC Excipient Information Package User Guide
6. Good Distribution Practices Guide
7. IPEC General Glossary of Terms and Acronyms
8. IPEC-PQG guide to Good Manufacturing Practices.
9. Technically Unavoidable Particle Profile (TUPP) Guide
10. Guide and Template for IPEC Quality Agreements (s)

11. The IPEC Significant Change Guide
12. QA-Manufacture's Quality Statement
13. QA Responsibility Table-Manufacturer's Template
14. QA Responsibility Table-Distributor's Template
15. PDA-IPEC Technical Report

INTERNATIONAL PHARMACEUTICAL EXCIPIENTS COUNCIL INDIA

DRUG REGULATORY SYSTEM IN INDIA

The Indian constitution lists Drugs and Health concurrently as one of its most important segments that is governed by both Centre and State governments under Drugs and Cosmetics Act 1940 & Rules 1945[10].

• Various bodies control Drugs and Health regulations in India:

REGULATORY AGENCY FRAMEWORK– INDIA

Flow chart 2: Framework for regulatory agencies.

NEW DEVELOPMENTS

For pharmaceutical crude materials, a microbial cut-off test is used.

• Imported medicine goods are compliant with India's Drug &Cosmetic Act 1940 and Rules 1945.
• IP consistency for imported excipients - the equivalent should be included in the COA
• Nutraceuticals /dietary-health-supplements (DS-HS) regulatory status in India

Microbial breaking point test for Pharma crude materials

Anyway, excipient producers – a significant number of which are not connected to the FDA or IP don't know about this necessity. Not all excipients are in danger for miniature development and testing each part isn't viable to those (There aren't enough internal or external labs to meet the influx of interest.) Furthermore, the expense of doing this study would significantly raise the cost of excipients, putting a strain on the budget which would very probably be provided to pharmaceutical clients. The production of drugs will be harmed if approaching crude materials are rejected due to a lack of this requirement. Routine testing is not necessary if you can obtain experimentally credible data from your supplier proving why there shouldn't be a microbiological concern, according to the USP/PhEur regulation. That's how the USP and PhEur do it, and it's how it's usually done to show consistency, along with some kind of intermittent watch that can be put up as a check. Currently, the pharmaceutical industry is decoding it using the USP/PhEur techniques, although there have been a few instances when customers' interest in this consistency has gotten in the way of the guideline. IPEC India wants to pursue this, in addition to comments made IPEC Americas and IPEC Federation, USP, a few pharma-related organisations, and a number of excipient manufacturers producers who have agreed to participate in discussions with the IP Commission.[11].

5.2.2 Imported drug products must comply with India's Drugs and Cosmetics Act and Rules.

CDSCO issued a notification on March 4, 2014, stating that all medicinal goods excipients that are imported into India must conform with the D&C Act 1940/Rule 1945, which enforces the Expiry date rather than the Retest or reconsideration technique that is usually employed by excipients.

- It gives the sense that this new notification may be used to put plans in place. Since any fixing in a medication is defined as a medication under this Act, it is unclear whether expiry dates are required or whether this will lead to a skewed understanding by Pharma organisations due to a lack of clarity in the notification, but India is changing its methods of authorising guidelines in some way.
- Excipients are exempt from the notification for the time being.due to concerns expressed by industry with Customs and Regulatory experts.
- However, a DCGI rule would be preferable in this case.

IP conformity for Imported Excipients:

When an Indian Pharmacopeia monograph is available, establishing a requirement from users and regulators to assure IP compliance for an excipient.

- There are differences in translation across controllers and, more crucially, between clients.
- An excipient manufactured outside of India will meet the requirements for the country of origin, but it will not be registered with the Indian FDA because there is no tool for confirming foreign producers.
- The excipient may agree to IP Testing principles, but it lacks Indian GMP conformity affirmation to guarantee or evaluate the item as IP.

INDIA IPEC FORMATION

IPEC India became a non-profit organisation in January 2014. Currently, a member of the IPEC Federation.

IPEC INDIA – STAKE HOLDERS

Creating to be a well – perceived free affiliation adequately making mindfulness bridging the gap between government and non-government; existing guidelines and the next set of guidelines to be developed; coordinating the gaps in principles and guidelines among neighbours and throughout the world. Create, execute, and advance wilful direction and different projects for the world drug industry that are intended to guarantee proceeded with accessibility of excipients and related segments for completed items that fulfil the most elevated suitable guidelines for quality, security and usefulness all through their assembling cycle and store network; Encourage and assist the pharmaceutical industry, the Food and Medication Administration, the Indian Pharmacopeia , and other general well-being excipients, as well as compendial principles for medication excipients. By supporting, educating, and assisting administrative professionals, you may make a difference in their lives.Industry associations and logical bodies attempting to propel general wellbeing on issues identifying with the production, dispersion, use, and usefulness of excipients [12].

GOALS OF IPEC INDIA

IPEC INDIA WILL

- Examine existing standards and aggressively build new ones advance extra logically stable, hazard-based guidelines through inside turn of events and impacting outer associations.
- Maintain and create outside communitarian connections and set up new ones as proper to meet individuals' targets.
- Ensure it proceeded with reasonability by giving the important assets to accomplish its destinations.
- Implement proper standard checking of the outside variables affecting IPEC and excipients, to advise the participation and other suitable associations.
- Develop an advancement and correspondence program by methods for classes, online courses, workshops, cooperation in conferences, displays to inform government, business, the media, and the general public on important topics and accomplishments
- Create mindfulness among excipient makers, merchants and clients about the different IPEC Guidelines for the Industry
- Develop, advance and support a science-based hazard the executive's way to deal with lifecycle the board that is fitting to the upkeep of purchaser security and feasible for the store network.

IPEC INDIA ORGANIZATION STRUCTURE

Numerous worldwide excipient makers elude the intentional for making their own things; IPEC-PQG Good Manufacturing Practices and IPEC Good Distribution Practices advisors are available. IPEC India is upholding the utilization of these rules to our enrolment. EXCIPACT and ANSI are other non-government private associations that can give GMP accreditations to excipient makers dependent on fruitful reviews directed by approved bodies. The administrative climate is consistently changing and for instance a "lawful" prerequisite and mandate for excipients putting the onus of excipient quality on the medication item maker is the EU Falsified Medicines (Directive 2011/62/EU). As an outcome of this, rules were given by the European Commission (2015/C 95/02) which came into

power since March 2016. The rules require the promoting approval holder to guarantee that figured danger evaluation to decide proper great assembling practice for excipients [13].

CONCLUSION

Excipients are, in the vast larger part of cases, not made explicitly for drug use. Most drug excipient makers supply fewer than 10% of the absolute creation of that specific material for drug use. Excipient item portfolio comprises of several items contrasting in science, source and usefulness and they are utilized in various applications. The times of treating excipients like items and getting them without completely qualifying the source and the whole dissemination chain have passed by as good manufacturing practise (GMP) guidelines requests to guarantee nature of different materials utilized in the assembling cycle. The current commitment gives a review about the current proceeds onward good manufacturing practice necessities for drug excipient and approach for capability of drug excipient makers. All the Excipients fabricated ought to conform to International pharmaceutical excipients council rules. A few medications generally used to treat youngsters contain hurtful excipients in sums that may surpass the Acceptable daily intake (ADI) in grown-ups. Clinicians should know about this to endorse proper treatment in this populace. So, its pharmaceutical companies' responsibility to maintain the good quality and safety of the excipients and follow the guidelines.

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CONFLICTS OF INTEREST

There are no conflicts of interest.

ABBREVIATIONS

IPEC- International Pharmaceutical Excipients Council
API- Active pharmaceutical ingredient's
FDA – Food and drug administration
GMP – Good manufacturing practice
EIP – Excipient Information Package
IID – Inactive Ingredient Database
ICH – International Conference on Harmonization
PDG – Pharmacopoeial Discussion Group
MHFW – Ministry of Health and Family Welfare
CDSCO – *Central Drugs Standard Control Organisation*
ADI – Acceptable daily intake

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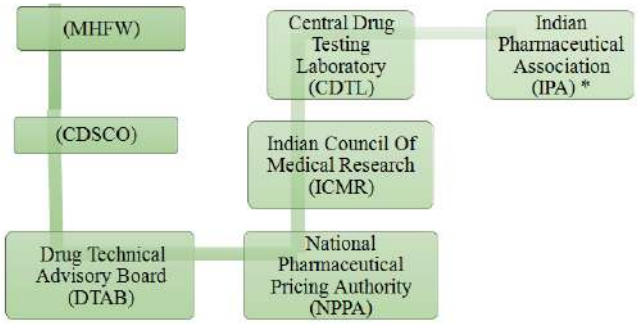
Table 1: Formation of IPEC Events

Years	Events
1991	Creation of IPEC Americas
1992	Officially IPEC Europe was created on 7th April
1992	Creation of IPEC Japan
1997	Publication of Guidelines for Safety Evaluation of Excipients.
1998	IPEC Europe launches IPEC website (first website)
2000	Publication of GMP Audit Guideline
2003	First WHO Good Trade and Distribution Practice Guidelines.
2006	Publication of first GMP Guide with PQG
2008	Creation of IPEC CHINA
2010	Creation of IPEC Federation
2012	Creation of EXCIPACT
2014	Creation of IPEC India

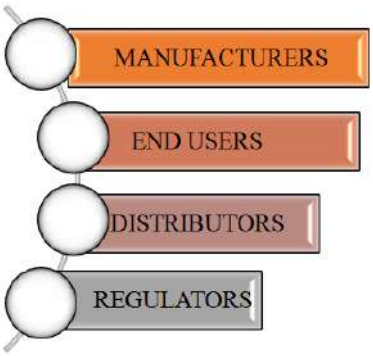
Figure 1:Search in the Inactive Ingredient Database

Figure 2: source - https://ipec-federation.org/

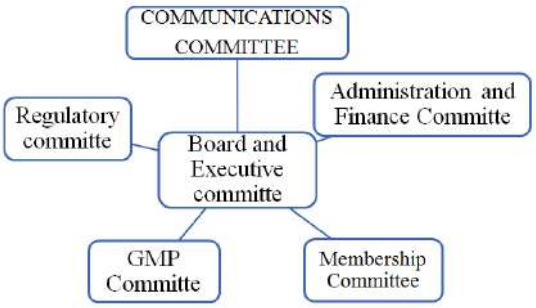


Flow chart 1: Drugs and health regulations in India are regulated by a number of organisations.

Flow chart 2: Framework for regulatory agencies



Flow chart 3: Stake holders of IPEC INDIA



Flow chart 4: IPEC India Organization Structure.

RESEARCH ARTICLE

Analysis of an M\M\co Queue with Encouraged Arrivals and Catastrophes

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ABSTRACT

This paper analyses a Markovian queueing system with encouraged arrivals and infinite server. Analytical solutions are derived for the transient probabilities of the system size using continued fraction methodology and confluent hypergeometric functions. The behaviour of the system during steady state is discussed and the factorial moments are deduced for the system.

Keywords: Transient analysis; Steady-state Analysis; Catastrophes; Continued fraction; Confluent hypergeometric functions. Mathematics Subject Classification 60K25.

INTRODUCTION

Queueing is a prevalent phenomenon in our daily lives. We all experience waiting in a queue before receiving service in a ticket counter, in a library, in a coffee shop and also on a phone. Even though waiting to get served is annoying, no one can escape waiting in a line. Hence queueing theory has become a fascinating area of research in recent years due to their widespread applicability in many real time situations. Queueing models play a significant role in the performance analysis of telecommunication systems, industrial engineering and computer networks. These communication systems can be modelled into queueing systems and can be analysed to achieve high profit goals and productivity.

To make a queueing system efficient, they can be modelled mathematically in order to monitor the arrival process and to study the characteristics of waiting lines to reduce the delay time.The arrival and service rates of a queueing system depends on the conditions of the system and many researchers study the queueing structures with different

arrival and service rates. Massey and Whitt [1]used random arrivals while constructing a Poisson arrival location model for networks of services. The transient behaviour of an $M\backslash M\backslash 1$ queue having customer's impatience with threshold and retention of reneging customers was studied by Sharma et al. [2]. Nazarov et al. [3]constructed a mathematical model of a queueing system with N-servers for repeated customers and delayed feedback. They used asymptotic analysis method to find the joint probability distribution of the number of occupied servers in the system. A fluid queue driven by a single server queue was analysed by Sophia and Muthu Deepika [4]in which the arrival and service rates follow chain sequence rates. They found the buffer content distribution along with averages using continued fraction technology. Ezeagu et al. [5]employed the fourth order Runge-Kutta method to find the time-dependent state probabilities for a finite capacity M/M/1 queueing system with working breakdowns and recovery policies.

In many real time situations, the arrival rate of customers increases due to various reasons and exceeds the normal capacity. This type of customers who increase the arrival rate are termed as encouraged arrivals (Som and Seth [6]). In an infinite server queueing system, the customers get service immediately upon arrival and hence it is of great interest to study this queueing system with various arrival rates. A network of infinite-server queueing system was studied by Boxma et al. [7] where the arrival rate is a vector-valued linear transform of a multivariate generalized shot-noise process. In the reviewing article on infinite server queues Worthington et al. [8] in their paper say that the infinite server models often serve as good and useful approximations for multi-server queueing systems. They also specify that the infinite server model serves as the basis for important offered-load analysis, which characterizes the total load faced by the system and serves as the basis for much useful engineering analysis.

The notion of catastrophe plays a vital role in computer and manufacturing networks. In a computer network, if a processor is affected by a virus it transmits the virus to other processors and make the system inactive. This can be modelled as queueing systems with catastrophes. Ascione et al. [9]considered a fractional $\boldsymbol{M\backslash M\backslash 1}$queue with catastrophes and analysed the transient behaviour of the queue in the presence and absence of catastrophes. Balasubramanian et al. [10] studied the effect of catastrophes in a queueing system with voice over internet protocol and derived the study state distributions using probability generating function. Transient system size probabilities were determined by Yaseen and Tarabia [11]for a queueing system with Balking and Reneging Subject to Catastrophes and Server Failures. This paper studies the transient behaviour of an infinite server queue with encouraged arrivals and catastrophes. The queueing model under consideration is explained in the following section 2. Section 3 deals with the transient analysis. Steady state analysis, factorial moments and mean system size are discussed in Section 4.

Model description

This paper analyses the behaviour of an infinite server queue with encouraged arrivals and catastrophes. Let β denotes the percentage increase in the customer's arrival rate and the arrival pattern of customers follows Poisson distribution with parameter λ. The service rate of the customers follows Exponential distribution with mean $\frac{1}{\mu}$ and the customers are served on First Come First Served basis. In addition, the catastrophes also occur at the service facility according to a Poisson process with rateα.Whenever a catastrophe occur at the service facility all the customers are wiped out, and the server is ready for service when a new customer arrives. Let $P_n(t)$ be the probability that there are n customers in the system at time t. The arrival and service rates when there are n customers in the system are

$\lambda_n = \lambda(1+\beta), n = 0,1,2,\ldots$ and $\mu_n = n\mu, n = 1,2,3,\ldots.$

For the queueing system under consideration the Chapman-Kolmogorov forward differential-difference equations are

$$\frac{dP_0(t)}{dt} = -\lambda(1+\beta)P_0(t) + \mu P_1(t) + \alpha(1-P_0(t))(1)$$

and $\frac{dP_n(t)}{dt} = \lambda(1+\beta)P_{n-1}(t) - (\lambda(1+\beta) + n\mu + \alpha)P_n(t) + (n+1)\mu P_{n+1}(t)$,
$n = 1,2,3, \ldots,(2)$

with the boundary conditions
$P_0(0) = 1$ and $P_n(0) = 0,\ n = 1,2,3, \ldots.$

Transient analysis of an $M\backslash M\backslash\infty$ queue with encouraged arrivals

In this section, the state probabilities$P_n(t),\ n = 0,1,2, \ldots$ of the queueing model are determined by taking Laplace transforms of the differential equations. Then by employing continued fraction methodology and by using confluent hypergeometric functions, explicit expressions are obtained for the transient state probabilities. In the sequel, let$P^*(s)$denote the Laplace transform of $P(.)$.

The confluent hypergeometric functions also known as Kummer functions is defined by

${}_1F_1(a;c;z) = \sum_{k=0}^{\infty} \frac{(a)_k z^k}{(c)_k k!}$, where$a, c, z \in C$ *and* ${}_1F_1(0;c;z) = 1$. $(a)_k$ is known as the Pochhammer symbol, defined by$(a)_k = \frac{\Gamma(a+1)}{\Gamma(a-k+1)}$, $k = 0,1,2, \ldots.$

We use the following identities to rewrite the continued fraction expressions as Kummer functions.

$$\frac{{}_1F_1(a+1;c+1;z)}{{}_1F_1(a;c;z)} = \frac{c}{c-z+}\frac{(a+1)z}{c-z+1+}\frac{(a+2)z}{c-z+2+}\ldots. \text{(I)}$$

$$c\frac{{}_1F_1(a+1;c+1;z)}{{}_1F_1(a;c;z)} - (c-z) = \frac{(a+1)z}{c-z+1+}\frac{(a+2)z}{c-z+2+}\frac{(a+3)z}{c-z+3+}\ldots. \quad \text{(II)}$$

$$\sum_{k=0}^{\infty} \frac{(a)_k y^k}{(c)_k k!}\ {}_1F_1(a+k;c+k;x) = {}_1F_1(a;c;x+y) \quad \text{(III)}$$

Taking Laplace transform of (1)and (2)and by using the boundary conditions, we get
$sP_0^*(s) = 1 - \lambda(1+\beta)P_0^*(s) + \mu P_1^*(s) + \alpha(\frac{1}{s} - P_0^*(s))(3)$
and

$$(s + \lambda(1+\beta) + n\mu + \alpha)P_n^*(s) = \lambda(1+\beta)P_{n-1}^*(s) + (n+1)\mu P_{n+1}^*(s).(4)$$

From (3), aftersome algebraic manipulation $P_0^*(s)$is obtained as

$$P_0^*(s) = \frac{1+\frac{\alpha}{s}}{[s+\lambda(1+\beta)+\alpha] - \frac{\mu P_1^*(s)}{P_0^*(s)}}. \quad (5)$$

Similarly, from (4) we obtain

$$\frac{P_n^*(s)}{P_{n-1}^*(s)} = \frac{\lambda(1+\beta)}{[s+\lambda(1+\beta)+n\mu+\alpha] - \frac{(n+1)\mu P_{n+1}^*(s)}{P_n^*(s)}}.(6)$$

By iteration, from (5)and(6), we get a continued fraction expression for $P_0^*(s)$ as
$P_0^*(s) = (1+\frac{\alpha}{s})\frac{1}{[s+\lambda(1+\beta)+\alpha] - \frac{\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+\mu+\alpha] - \frac{2\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+2\mu+\alpha] - \ldots}}}.$
Now, by using the identity (I), the above equation can be written in confluent hypergeometric function asfollows

$$\frac{s+\alpha}{{}_1F_1\left(1;\frac{s+\alpha}{\mu}+1;\frac{-\lambda(1+\beta)}{\mu}\right)}$$

$$= s + \alpha + \lambda(1+\beta) - \frac{\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+\mu+\alpha] - \frac{2\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+2\mu+\alpha] - \frac{3\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+3\mu+\alpha]-\dots}}},$$

which after simplification modifies to

$$P_0^*(s) = \frac{\left(1+\frac{\alpha}{s}\right)\ {}_1F_1\left(1;\frac{s+\alpha}{\mu}+1;\frac{-\lambda(1+\beta)}{\mu}\right)}{s+\alpha}.$$

Successive iteration of (6) gives

$$\frac{P_n^*(s)}{P_{n-1}^*(s)} = \frac{\lambda(1+\beta)}{[s+\lambda(1+\beta)+n\mu+\alpha] - \frac{(n+1)\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+(n+1)\mu+\alpha] - \frac{(n+2)\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+(n+2)\mu+\alpha]-\dots}}}$$

as before by using the identity (I) in the above equation, we get

$$(s+\alpha+n\mu)\frac{{}_1F_1\left(n;\frac{s+\alpha}{\mu}+n;\frac{-\lambda(1+\beta)}{\mu}\right)}{{}_1F_1\left(n+1;\frac{s+\alpha}{\mu}+n+1;\frac{-\lambda(1+\beta)}{\mu}\right)} - (s+\alpha+n\mu+\lambda(1+\beta))$$

$$= \frac{-(n+1)\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+(n+1)\mu+\alpha] - \frac{(n+2)\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+(n+2)\mu+\alpha] - \frac{(n+3)\mu\lambda(1+\beta)}{[s+\lambda(1+\beta)+(n+3)\mu+\alpha]-}} \dots\dots$$

which ultimately gives

$$\frac{P_n^*(s)}{P_{n-1}^*(s)} = \frac{\lambda(1+\beta)\ {}_1F_1\left(n+1;\frac{s+\alpha}{\mu}+n+1;\frac{-\lambda(1+\beta)}{\mu}\right)}{(s+\alpha+n\mu)\ {}_1F_1\left(n;\frac{s+\alpha}{\mu}+n;\frac{-\lambda(1+\beta)}{\mu}\right)}. \quad (7)$$

(7) on successive iteration yields $P_n^*(s)$ as follows

$$P_n^*(s) = \frac{\left(1+\frac{\alpha}{s}\right)(\lambda(1+\beta))^n\ {}_1F_1\left(n+1;\frac{s+\alpha}{\mu}+n+1;\frac{-\lambda(1+\beta)}{\mu}\right)}{(s+a)(s+\alpha+\mu)(s+\alpha+2\mu)\dots(s+\alpha+n\mu)}. \quad (8)$$

Now by using the definition of Kummar functions $P_n^*(s)$ can be expressed as

$$P_n^*(s) = \frac{\left(1+\frac{\alpha}{s}\right)(\lambda(1+\beta))^n}{(s+a)(s+\alpha+\mu)(s+\alpha+2\mu)\dots(s+\alpha+n\mu)} \times \left[\sum_{k=0}^{\infty} \frac{(n+1)(n+2)(n+3)\dots(n+k)\left(\frac{-\lambda(1+\beta)}{\mu}\right)^k}{k!\left(\frac{s+\alpha}{\mu}+n+1\right)\left(\frac{s+\alpha}{\mu}+n+2\right)\left(\frac{s+\alpha}{\mu}+n+3\right)\dots\left(\frac{s+\alpha}{\mu}+n+k\right)}\right]$$

Thus $P_n^*(s)$becomes

$$P_n^*(s) = \left(1+\frac{\alpha}{s}\right)\sum_{k=0}^{\infty}\frac{(n+k)!\,(-1)^k\,(\lambda(1+\beta))^{n+k}}{k!\,n!} \times \frac{1}{\prod_{i=0}^{n+k}(s+\alpha+i\mu)}$$

$$= \left(1+\frac{\alpha}{s}\right)\sum_{k=0}^{\infty}\frac{(n+k)!(-1)^k\,(\lambda(1+\beta))^{n+k}}{\mu^{n+k}k!n!}\sum_{i=0}^{n+k}\frac{(-1)^i}{i!(n+k-i)!(s+\alpha+i\mu)} \quad (9)$$

which on inversion yields $P_n(t), n = 0,1,2,\dots$ as

$$P_n(t) = \frac{e^{-\alpha t}}{n!}\left[\frac{\lambda(1+\beta)}{\mu}(1-e^{-\mu t})\right]^n e^{-\left(\frac{\lambda(1+\beta)}{\mu}(1-e^{-\mu t})\right)} + \frac{\alpha}{n!}\int_0^t e^{-\alpha y}\left[\frac{\lambda(1+\beta)}{\mu}(1-e^{-\mu y})\right]^n e^{-\left(\frac{\lambda(1+\beta)}{\mu}(1-e^{-\mu y})\right)}dy \quad (10)$$

Thus equation (10) provides the transient probabilities of the states $n = 0,1,2,$ ...for the system under consideration.

Stationary Analysis and Moments

The steady state probabilities when the system is in equilibrium are obtained by applying Tauberian theorem. Multiplying (9) by s on both sides and taking the limit as $s \to 0$, we get

$$\lim_{s\to 0} s\, P_n^*(s) = \alpha \sum_{k=0}^{\infty} \frac{(n+k)!(-1)^k\,(\lambda(1+\beta))^{n+k}}{\mu^{n+k}k!n!} \sum_{i=0}^{n+k} \frac{(-1)^i}{i!(n+k-i)!(\alpha+i\mu)}.$$

Consequently P_n is determined as

$$P_n = \alpha \sum_{k=0}^{\infty} \frac{(-1)^k\,(\lambda(1+\beta))^{n+k}}{\mu^{n+k}k!\,n!} \sum_{i=0}^{n+k} \binom{n+k}{i} \frac{(-1)^i}{(\alpha+i\mu)}$$

To determine the factorial moments for the model under consideration, Let us define

$$\Phi^*(x,s) = \sum_{n=0}^{\infty} P_n^*(s)x^n$$

Thus, using (8)

$$\Phi^*(x,s) = \left(1+\frac{\alpha}{s}\right) \sum_{n=0}^{\infty} \frac{(\lambda(1+\beta)x)^n \;\; {}_1F_1\left(n+1; \frac{s+\alpha}{\mu}+n+1; \frac{-\lambda(1+\beta)}{\mu}\right)}{(s+a)(s+\alpha+\mu)(s+\alpha+2\mu)\ldots(s+\alpha+n\mu)}$$

Now, by employing the identity (III) we get

$$\Phi^*(x,s) = \left(1+\frac{\alpha}{s}\right) \frac{{}_1F_1\left(1; \frac{s+\alpha}{\mu}+1; \frac{\lambda(1+\beta)(x-1)}{\mu}\right)}{s+\alpha}$$

$$= \left(1+\frac{\alpha}{s}\right) \sum_{k=0}^{\infty} \frac{(\lambda(1+\beta)(x-1))^k}{\mu^k} \sum_{i=0}^{\infty} \frac{(-1)^i}{i!\,(k-i)!\,(s+i\mu+\alpha)}$$

which on inversion gives

$$\Phi(x,t) = e^{-\left[\alpha t + \frac{\lambda(1+\beta)}{\mu}(1-x)(1-e^{-\mu t})\right]} + \alpha \int_0^t e^{-\left[\alpha u + \frac{\lambda(1+\beta)}{\mu}(1-x)(1-e^{-\mu u})\right]} du.$$

Now, differentiating the above equation with respect to x and setting $x = 1$ gives the mean of the queueing system under consideration as

$$E(t) = \frac{\lambda(1+\beta)(1-e^{-(\alpha+\mu)t})}{\alpha+\mu}$$

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RESEARCH ARTICLE

Novel Method of Increasing the Maximum Frequency Shift in Phase Modulation by Electro-Optic Crystal with Multiple Rotation Technique

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ABSTRACT

There are several uses of electro-optic crystal in optical modulation. Different types of modulations are conducted by this modulator for transmission of optical data through wave guide. Here in this chapter I propose a novel concept for using electro-optic material (like$LiNbO_3$) for modulating an optical signal by a low frequency message signal for increasing band width of the signal. An audio signal with smaller amplitude also can be well modulated by the proposed mechanism.

Keywords: Electro-optic , Optical modulation, Multi passing technique, Phase modulation, Electro-optic crystal.

INTRODUCTION

Electro-optic materials can use its non-linearity for developing several all-optical processing systems. It is massively used in several optical modulation schemes, integrated optical circuits, optical shutters etc [1,2]. In those systems $LiNbO_3$ (Lithium Niobate), $LiIO_3$ (Lithium Iodate), KDP,ADP etc. are well recognized.$LiNbO_3$ waveguide has been used in microwave modulation also. Yi Liao et~al proposed the scheme of using micro-engineered $LiNbO_3$, which results 15Gb/S signal modulation [3]. Again D. Janner et~al proposed the scheme of using micro-engineered $LiNbO_3$ for waveguide electro-optic (e.o) modulation [4]. Simultaneous amplitude and phase modulation in electro-optic modulator were proposed by B.J Cusack et~al[5] .In all the above cases e.o modulator was successfully used for modulation of a low (audio) to high (microwave) frequency message signal. It is known also that for successful modulation the bandwidth should be increased properly and to increase the bandwidth of phase modulation amplitude of the signal wave should be increased accordingly. This is a power consuming issue. In last few decades a large no .of works have been reported where electro-optic modulators have been used successfully for modulating an electronic/electrical signal by a light based carrier signal [6-16]. In this context I propose a new concept of increasing the bandwidth in phase modulation not by increasing the amplitude of the signal. Here the multiple

rotation of the beam through the modulator takes the role of increasing the bandwidth of phase modulation instead of increasing other power consuming factors.

Real life application of the method

Electro-optic modulators can extend several functions which help the exploitation of optics in communication, data processing, image processing etc .For the control of phase of a signal, for the purpose of modulation with light as carrier wave, in integrated optics, in optical switching and in several other applications electro-optic modulator has the potential applications[6,7]. Its speed of operation is also very high, and for this reason it can be used in microwave modulation. Optical shutters, spatial light modulators can also be developed by $LiNbO_3$ based electro-optic modulators. There also lies some applications using electro-optic modulators. Variable capacitance of the tank circuit of a concerned frequency modulation system can be implemented by the electro-optic modulator. Here in this communication we propose an optical process for reducing the V_π voltage of the modulator. If the V_π is reduced it can be used very successfully in optical modulations.

Phase modulation in electro-optic crystal

The simplest kind of EOM consists of a crystal like Lithium Niobate, whose refractive index is a function of the strength of the local electric field. If Lithium Niobate is exposed to an electric field, light will travel more slowly or fast through it depending of the external applied electric field and the direction of radiation. But the phase change of the light leaving the crystal is directly proportional to the length of material through which the light passes. Hence the phase change of a laser light in an EOM can be controlled by changing the electric field in the crystal (fig 1). In a phase modulators an electric field modulates the phase change of a laser beam emitted through the crystal. The polarization of the input beam should be selected properly such that it does not change during the propagation of the light through it.

Method of increasing the frequency deviation in phase modulation

First an electro-optic modulator is taken which is connected with an external modulating a.c signal $V_m = V_0 \sin \omega_m t$ along its Z-axis (fig 2). The length of the modulator is d along its the light passing along the Z axis and Y direction .Now a light wave polarized along 45^0 to its Z axis in X-Z plane is passed through the modulator along Y direction. The refractive index of component of light polarized along X direction is $n_y = n_0 - \frac{1}{2} n_0^{\,3} r_{13} \frac{V_0 \sin \omega_m t}{d}$ .The expression of its electric field after passing through the length $d_1$along Y direction of the modulator is

$$E_1 = E_0 \sin(\omega t + k_0 n_y d_1 + \phi_1)$$

$$= E_0 \sin(\omega t + k_0 (n_0 - \frac{1}{2} n_0^{\,3} r_{13} \frac{V_0 \sin \omega_m t}{d}) d_1 + \phi_1)$$

$$= E_0 \sin(\omega t + k_0 (n_0 - \frac{1}{2d} n_0^{\,3} r_{13} V_0 \sin \omega_m t) d_1 + \phi_1)$$

$$E_1 = E_0 \sin(\omega t + k_0 n_0 d_1 - \frac{1}{2d} k_0 n_0^{\,3} r_{13} V_0 \sin(\omega_m t) d_1 + \phi_1) \quad (6.1)$$

K_0 is the free-space wave numbers of the used light and r_{13} is the e.o coefficient of the material.

As the wave is now passed again through the modulator and after exit from the modulator the expression becomes

$$E_2 = E_0 \sin(\omega t + k_0 n_0 d_1 - \frac{1}{2d} k_0 n_0^{\,3} r_{13} V_0 \sin(\omega_m t) d_1 + \phi_1 + k_0 n_{13} d_1 - \frac{1}{2d} n_0^{\,3} r_{13} V_0 \sin \omega_m t d_1 + \phi_2) \quad (6.2)$$

Here ϕ_1 and ϕ_2 are the additional phases introduced in the expression during passage of the light outside the modulator.

So

$$E_2 = E_0 \sin(\omega t + 2k_0 n_0 d_1 - \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \sin(\omega_m t) d_1 + \phi_1 + \phi_2) \quad (6.3)$$

Similarly after the 3rd cycle the expression becomes

$$E_3 = E_0 \sin(\omega t + 3k_0 n_0 d_1 - \frac{3}{2d} k_0 {n_0}^3 r_{13} V_0 \sin(\omega_m t) d_1 + \phi_1 + \phi_2 + \phi_3) \quad (6.4)$$

Equation 6.3 gives the angular part of ϕ as

$$\phi = \omega t + 2k_0 n_0 d_1 - \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \sin(\omega_m t) d_1 + \phi_1 + \phi_2 \quad (6.5)$$

Differentiating with respect to ϕ the frequency becomes

$$\omega_0 = \frac{d\phi}{dt} = \omega - \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \cos(\omega_m t) \omega_m d_1 \quad (6.6)$$

Hence the minimum frequency is

$$\omega_{0\min} = \omega - \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 (+1) \omega_m d_1 \quad (\text{putting } \cos \omega_m t = +1)$$

$$\omega_{0\min} = \omega - \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \omega_m d_1 \quad (6.7)$$

And maximum frequency is

$$\omega_{0\max} = \omega - \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 (-1) \omega_m d_1 \quad (6.8)$$

$$\omega_{0\max} = \omega + \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \omega_m d_1 \ (\text{putting } \cos \omega_m t = -1) \quad (6.9)$$

So the band width is

$$\Delta\omega_2 = \omega_{0\max} - \omega_{0\min} = \omega + \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \omega_m d_1 - \omega + \frac{2}{2d} k_0 {n_0}^3 r_{13} V_0 \omega_m d_1$$

$$= \frac{4}{2d} k_0 {n_0}^3 r_{13} V_0 \omega_m d_1 \quad (6.10)$$

$$\Delta\omega_2 = 2k_0 {n_0}^3 r_{13} V_0 \omega_m d_1 \quad (6.11)$$

For passing of the light through the electro optic modulator 2nd times the band width is increased 2 times.

Similarly after differentiating angular part of the equation (6.5) with respect to't' one can get the frequency of the light obtained from the electro-optic modulator for passing the light 3 times.

Here the band width $\omega_{\max} - \omega_{\min} = \Delta\omega_3$

$$\Delta\omega_3 = 3k_0 {n_0}^3 r_{13} V_0 \omega_m \frac{d_1}{d} \quad (6.12)$$

This is 3times than that of the band width with respectively that of the light passed single time.

Now if the material is $LiNbo_3$ then putting the values of k_0, n_0, r_{13}, ω_m, V_0, d_1, d;

(Where $k_0 = \frac{2\pi}{\lambda_0}$ for $\lambda_0 = .633\times10^{-6}$ μm, n_0=2.297, $r_{13}=8.6\times10^{-12}$ m/V, V_0 is the source voltage=100volt,d_1= $.25\times10^{-3}$ m.d=10×10^{-3}m. $\omega_m = 1MHz = 10^6$ Hz.)

$$\Delta\omega_1 = k_0 n_0^{\ 3} r_{13}\omega_m V_0 \frac{d_1}{d}$$

=2555Hz

Similarly from equation (6.10) we get

$$\Delta\omega_2 = 2\times\Delta\omega_1 = 2\times2555 = 5110Hz$$

And from equation (6.11) we get

$$\Delta\omega_3 = 3\times\Delta\omega_1 = 3\times2555 = 7665Hz$$

After 3times rotation it is seen that the band width is till far lower than 1MHz.Now rotating the single multiple time the band width may be increased to 1MHz not increasing V_0,d_1,d etc.

CONCLUSION

It is concluded that the bandwidth of a signal passing through the electro-optic modulator increases for multi passing of the beam through the modulator. For n time passing of the beam the bandwidth increases also n times. The phenomenon is the very much helpful for analog optical communication, which requires high band width and also in frequency conversion.

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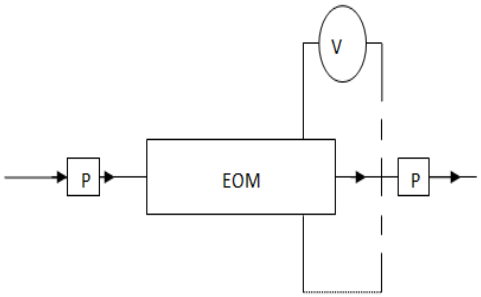

Fig 1: Phase modulation scheme by an electro-optic modulator, (P is suitable polarizer)

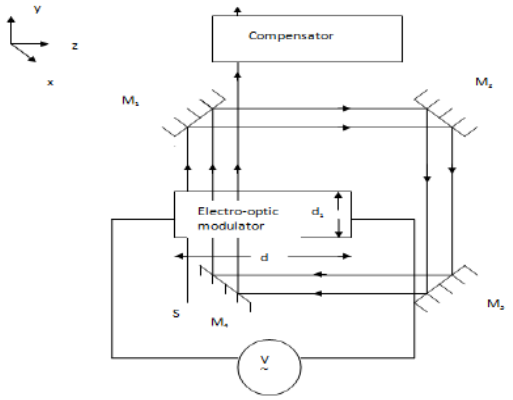

Fig-2 Multiple rotation of a beam through electro-optic modulators

RESEARCH ARTICLE

Study of the influence of fodder and soil on the availability of various Macro and Micro-Minerals in Lactating cows in Cuddalore, TamilNadu

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ABSTRACT

A study was conducted in the Cuddalore district of Tamil Nadu to assess the influence of fodder and soil on the status of some macro and micro- minerals in lactating cows. Feeds and fodder samples were collected from 17 representative villages of the district for analysis of macro and micro-minerals. Calcium content in Groundnut cake (0.17%) and crushed maize (0.03%) was found to be below the critical level (0.30%). The phosphorus content in concentrate ingredients was high (0.32-0.67%) but low in dry roughages (0.06-0.20%). Feeds and fodder were found to be adequate in magnesium (0.40%), sodium (0.29%) and potassium (1.15%). Straws were found to be deficient in sulphur (0.16%). Green roughages were good source of copper (12.31 ppm). Wheat straw was found to be low in zinc (19.71 ppm) but comparatively high in manganese (47.88 ppm) and iron (630.24 ppm). Lucerne and was found to be rich source of cobalt (>0.35 ppm). Selenium (0.68 ppm) was present in appreciable quantities in most of the feedstuffs. Lactating cows were also found to be excess in energy and crude protein (70%), whereas, calcium and phosphorus were deficient in the ration (65%). Ration of lactating cows was found to be deficient in Ca, P, S, Cu, Zn and Co. Supplementing the deficient minerals through area specific mineral mixture could alleviate the deficiency and improve productivity and reproduction efficiency of lactatingcows.

Keywords: Calcium, Phosphorus, Copper, Zinc, Selenium, Lactating cows.

INTRODUCTION

The importance of minerals in regulating biological systems, growth, production and reproduction is well documented [1], however, livestock in India do not receive mineral/vitamin supplements except for common salt and calcite powder [2]. Hence, dairy animals depend on forages for their mineral requirements [3]. A number of researchers in the world have reported high incidences of forage and blood serum samples below the critical levels for different mineral elements, especially copper (Cu), zinc (Zn) and phosphorus [4]. Soils from all over country are getting depleted for one or more mineral elements in soil, plants and animals [5]. The quantity of minerals, thus, present in forages may not be sufficient for optimum growth, milk yield and reproduction efficiency of dairy animals [3]. In order to avoid macro and micro-minerals imbalances in the ration, a study on assessment of mineral status of lactating cows was undertaken in Cuddalore district of Tamil Nadu.

MATERIALS AND METHODS

Sampling Procedures

One or two villages from each taluka were selected at random for taking representative samples of feeds, fodders and hair. Total area of Sabarkantha district is 36787sq.km., distributed into 10talukas, having 896villages. The district is having annual rainfall of 1086.4 mm, Latitude is $15^0\ 5^{11}$ /$11^0\ 11^{11}$ and $12^0\ 35^{11}$ N, Longitude is $78^0\ 38^{II}$ to $80^0\ 00^{11}$ and Altitude 4.6m MSL.. Within the village, help was sought from village milk producers and district animal husbandry officer for identification of 4 to 5 farmers. The recorded parameters were number of livestock, land area, irrigation facilities, fodder and other crops being grown etc. In identification of farmers, land location was considered essentially, one each from northern, eastern, western and southern direction to cover soil types on each side of the selected village. Further information regarding the amount and types of feeds and fodder being offered to the animals, rate of daily feed intake, number of milch animals and milk yield were collected from individual farmer. Total intake was compared against the requirements on dry matter basis [6], so as to identify quantitative deficiency, sufficiency or evenexcess.

Sample Preparation and Analytical Methods

Composite samples of green fodder, dry fodder, concentrate ingredients and the compound cattle feed (concentrate mixture) were collected from all over the surveyed area. Green samples were dried in oven at 80^0C for 24 hours and subsequently ground (1mm). Ground samples of concentrate and fodder were stored in airtight bags until analysis. All the samples were analyzed for calcium (Ca), phosphorus (P), magnesium (Mg), sulphur (S), sodium (Na), potassium (K), copper (Cu), zinc (Zn), manganese (Mn), iron (Fe), cobalt (Co), selenium (Se) and molybdenum (Mo), using Inductively Coupled Plasma-Optical Emission Spectrometer (Perkin-Elmer, OPTIMA-3300 RL) [7]. The data were analyzed statistically as per Snedecor and Cochran [8].

RESULTS AND DISCUSSION

Crop residues were found to be the main source of roughage in the ration of animals. It was noticed that some of the farmers fed cultivated fodders like Lucerne (*Medicago sativa*), maize (*Zeamays*), jowar green (*Sorghum bicolor*), etc. Some farmers offered crushed maize, wheat bran alone or mixture of two. Feeding of groundnut cake, cottonseed cake and tapioca flour was also observed in some parts of district. Those farmers, who don't feed concentrate feed ingredients, were fed compound cattle feed to their cows, depending on the level of milk production. The use of common salt and mineral mixture supplementation was not a common practice in the surveyed area, except for therapeutic purpose.

Macro-Minerals Profile of Feeds and Fodders

The straws of wheat, jowar, paddy and groundnut were the main roughage source in the surveyed area (Table 1). The average Ca content ranged from 0.45to 1.30 percent in roughages, as compared to 0.03to 0.22 percent in concentrate feed ingredients. These findings are similar to the findings of Ramana*et al.* [9] and Yadav*et al.* [10]. P content in concentrates (0.25 to 0.67 %) was higher than roughages (0.06 to 0.20 %). Crushed grains were low in Mg as compared to cakes (Table 1). Sulphur content was found below critical level (<0.20%) in most of the straws and crushed grains (Table 1). Higher K level in green fodders (Table 1) may be due to its selective uptake from the soil and regular application of potash fertilizer in the soil [2]. Na content was low in some of the feed stuffs (Table 1).

Micro-Minerals Profile of Feeds and Fodders

Copper (Cu) content was found below the critical level (<8ppm) in all types of straws and concentrate ingredients except cottonseed cake and isabgol husk (Table **2**). Zinc (Zn) content was below critical level (<30ppm) in all the straws except paddy straw. Green fodders and oilcakes were found to be a better source of Zn, as compared to crushed grains. The Mn levels in the district ranged from 36.47-478.12 ppm in straws, 62.64-132.99 ppm in green fodders, 13.18-75.74 ppm in concentrate ingredients (Table **2**). Average Fe content was 769.43 ppm in roughage and 403.22 ppm in concentrates, showing adequacy of this mineral. Yadav*etal.* [10], Youssef*etal.* [11] and Mandal *et al*.[12] reported high Fe levels in forages. Cobalt in feeds and fodders ranged from 0.18 ppm to 0.71 ppm. Selenium content was adequate in all the feeds and fodder (Table **2**). High levels of molybdenum (>2 ppm) in forages could interfere with copper metabolism. The molybdenum levels as estimated in the samples of crop residues were within the safe limit. Most of the feedstuffs contained Mo level within the safe limit and gave Cu:Mo ratio wider than 5.0. Mo has gained more importance recently in animal nutrition, because of its inhibitory role on the other trace elements, particularly copper. Suttle [13] stated that a Cu:Mo ratio below 2.0 would be expected to cause conditioned Cu deficiency in cattle. Mo level at 5 to 6 ppm inhibits Cu storage and produce signs of molybdenosis [14]. Even 2 ppmor less Mo can be toxic, if forage Cu is sufficiently low [12]. In case of ruminants, Mo reacts with sulphur in the rumen and forms mono-, di-, tri- or tetra-thiomolybdates [13], making Cu unavailable for absorption and utilization[15].

Mineral Levels in Hair Samples of Lactating cows

Hair samples collected during survey were analyzed for the same minerals as in feeds and fodders. Mineral levels in hair must reflect the concentration and (or) activity of the certain minerals in other parts of the body and reflect dietary mineral status of animals [16] (Combs, 1987). The average levels of copper and zinc in hair were 6.28 and 73.51 ppm, respectively (Table **3**). When compared with critical levels for Cu (<10 ppm) and Zn (<100 ppm), 50 and 100 per cent animals showed sub-normal levels in hair samples indicating their dietary deficiency. It has been demonstrated in several studies that concentration of Zn in hair is correlated with dietary Zn intake. Studies have shown the level of Zn in hair on normal diet to be 120-150 ppm [1]. The selenium level of the hair of cattle is a useful indicator of both selenium deficiency and selenium toxicity. Most studies have shown that cattle with hair values consistently below 0.25 ppm probably need supplementation and that over 5 ppm may lead to clinical signs of selenosis. The average selenium level in hair samples was 2.82 ppm indicating the adequacy of the element in theration.

Daily Mineral Intake by Lactating cows

The daily intake of different minerals by a cow (450 kg body weight) yielding 10 kg milk (6% fat), with the prevailing feeding system in the surveyed area is presented in Table **4**. Since mineral mixture supplementation was not being followed so the intake of minerals through feeds and fodder was taken as index of total mineral supply and compared with the recommended requirements to know the dietary mineral adequacy/inadequacy. Ration of lactating cows was found to be deficient in Ca, P, S, Cu, Zn and Co. Hence, it is necessary to supplement these minerals in ration. It was observed that Mg, K, Na, Mn, Fe, Mo and Se in ration of animals were found to adequate. Supplementation of Cu and Zn in the form of chelates found to be effective in curing

problem of anestrous [17] and deficient trace minerals in the surveyed area need to be supplemented in chelated form for better bio-availability and retention in the animal system.

Nutritional Status of Lactating cows

In order of priority, available good quality feed resources are first allocated to lactating cows followed by dry pregnant, dry, heifers, growing calves and non-productive cows. In the surveyed area our observation indicates that, unlike metabolizable energy and crude protein, which were excess in the ration of more than 70 percent of the cows, calcium and phosphorus were deficient in the ration of about 65% of cows because of low mineral mixture supplementation, probably due to high costs. Dairy farmers in most developing countries often do not feed adequate quantities of mineral mixture to their animals due to non-availability, lack of knowledge on the benefits of feeding mineral mixtures or high cost [18]. Inview of this, supplementation of mineral mixture in the ration of dairy animals for improving production and reproduction efficiency need to be popularized.

Formulation of Area Specific Mineral Mixture

Information on the actual intake of each type of feeds and fodder for a particular level of milk production was collected from each of the individual dairy farmer, to calculate intake of various mineral elements against the requirement. Total mineral intake from feeds and fodder was compared against the requirements on dry matter basis (Table **5**), to identify quantitative deficiency and adequacy of minerals. Based on the degree of deficiency, area specific mineral mixture formulation was developed for the supplementing cows in the Cuddalore district of Tamil Nadu (Table **6**). To enhance the usefulness of mineral mixture, chromium was also incorporated in the formulation. Mg and S: 0.20 % of DM Intake, Copper: 10 ppm, Manganese: 40 ppm, Na: 0.18 % of DM Intake, Iron: 50 ppm, Cobalt: 0.50 ppm, K: 0.90 % of DM Intake, Zinc: 80 ppm, Selenium: 0.30 ppm, Cl: 0.25 % of DM intake, Iodine: 0.60 ppm, Chromium: 0.50 ppm.

CONCLUSION

It was evident from the present study that majority of lactating cows in Cuddalore district were deficient in Ca, P, S, Cu, Zn and Co. Therefore, it is necessary to supplement these minerals in the ration of cowss by formulating area specific mineral mixture, having highly bio-available mineral salts. Deficient trace minerals, except Co, may be supplemented in the form of chelates, for better bio-availability and improving productivity, reproductive efficiency and productive life of cows.

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Table 1: Levels of Macro Minerals in Feed and Fodder in Cuddalore District of Tamil Nadu

Particulars	Ca	P	Mg	S	K	Na
Critical level	**<0.30%**	**<0.25%**	**<0.20%**	**<0.20%**	**<0.9%**	**<0.06%**
Jowar straw (38)	0.57±0.02	0.19±0.01	0.49±0.02	0.14±0.01	1.11±0.08	0.07±0.01
Paddy straw (6)	0.45±0.01	0.09±0.01	0.34±0.01	0.15±0.01	1.56±0.04	0.40±0.04
Bajra straw (7)	0.45±0.01	0.16±0.02	0.75±0.12	0.17±0.02	2.23±1.35	0.23±0.12
Groundnut straw (10)	1.30±0.09	0.18±0.01	0.78±0.04	0.17±0.01	1.06±0.10	0.05±0.01
Sorghum Sudan grass (2)	0.70±0.04	0.32±0.01	0.40±0.06	0.27±0.02	4.30±0.02	0.04±0.01
Local grass (16)	1.11±0.18	0.25±0.02	0.59±0.06	0.31±0.02	2.06±0.30	0.78±0.26
Lucerne green (24)	1.67±0.10	0.31±0.10	0.43±0.03	0.39±0.02	1.42±0.20	0.34±0.04
Cottonseed cake (25)	0.17±0.01	0.67±0.01	0.39±0.01	0.29±0.01	1.34±0.03	0.04±0.01
Crushed maize (29)	0.03±0.01	0.32±0.01	0.13±0.01	0.12±0.01	0.40±0.01	0.03±0.01
Wheat bran (19)	0.07±0.01	0.32±0.01	0.14±0.01	0.16±0.01	0.44±0.01	0.03±0.01
Cattle feed (50)	0.79±0.11	1.12±0.04	0.66±0.02	0.37±0.03	1.10±0.03	0.47±0.06
Tapioca flour (5)	0.19±0.01	0.25±0.02	0.12±0.01	0.11±0.01	0.34±0.02	0.02±0.01
Whole cottonseed (2)	0.19±0.01	0.54±0.10	0.34±0.04	0.27±0.05	1.13±0.13	0.04±0.01
Hybrid- napier (2)	0.50±0.05	0.15±0.02	0.24±0.01	0.18±0.02	2.79±0.19	0.08±0.01
Jowar green (27)	0.58±0.05	0.23±0.01	0.50±0.05	0.16±0.01	1.27±0.10	0.06±0.01
Neem leaves (2)	1.00±0.21	0.18±0.01	0.26±0.04	0.39±0.03	2.70±0.69	0.07±0.03

Table 2: Levels of Micro Minerals in Feed and Fodder in Cuddalore District of Tamil Nadu

Particulars	Cu	Zn	Mn	Fe	Co	Mo	Se
Critical level	**<8ppm**	**<30ppm**	**<40ppm**	**<50ppm**	**<0.10ppm**	**>6ppm**	**<0.2ppm**
Wheat straw (58)	5.23±0.03	19.71±1.18	47.88±1.44	630.24±35.02	0.30±0.01	0.82±0.03	0.62±0.03
Jowar straw (38)	7.02±0.38	28.31±2.31	55.83±4.36	581.71±30.74	0.34±0.01	0.84±0.06	0.63±0.06
Paddy straw (6)	5.08±0.24	34.21±0.58	478.12±54.00	482.26±49.67	0.71±0.09	0.87±0.11	0.64±0.07
Bajra straw (7)	5.63±0.33	17.91±3.33	36.47±3.14	709.21±93.23	0.25±0.04	0.70±0.08	1.07±0.05
Groundnut straw (10)	5.40±0.78	23.36±3.46	44.64±3.73	1123.61±93.17	0.46±0.04	0.72±0.05	0.54±0.05
Bajri green (20)	11.85±0.69	49.07±3.22	65.58±3.13	741.33±43.91	0.42±0.01	1.72±0.39	1.05±0.12
Sorghum Sudan grass (2)	11.96±0.07	49.75±0.90	48.67±2.24	694.65±55.75	0.28±0.02	0.80±0.10	0.52±0.10
Local grass (16)	13.66±1.34	41.79±2.78	62.28±7.15	1118±197.03	0.59±0.08	0.79±0.10	0.76±0.10
Lucerne green (24)	13.30±0.94	35.96±3.20	41.82±1.54	571.46±38.69	0.38±0.03	2.10±0.47	1.02±0.13
Cottonseed cake (25)	8.98±0.15	40.97±0.72	15.32±0.25	135.80±10.54	0.37±0.01	0.83±0.07	0.68±0.05
Crushed maize (29)	4.50±0.05	24.18±1.38	13.18±2.34	106.90±17.06	0.21±0.01	1.63±0.07	0.41±0.05
Crushed wheat (19)	5.50±0.09	26.66±0.72	37.34±1.38	187.34±18.46	0.20±0.01	1.63±0.11	0.48±0.05
Cattle feed (50)	10.48±0.94	79.09±3.44	100.4±4.05	613.67±27.09	0.56±0.10	1.83±0.06	0.60±0.04
Tapioca flour (5)	4.02±0.14	22.51±1.36	41.74±0.86	1043.50±2.21	0.26±0.01	0.45±0.05	0.24±0.02
Whole cottonseed (2)	6.47±2.17	35.09±4.62	15.57±0.35	140.20±72.21	0.23±0.04	0.69±0.33	0.44±0.25
Hybrid napier (2)	10.08±0.20	32.39±1.05	132.99±2.81	629.79±8.91	0.35±0.03	0.68±0.03	0.67±0.03
Jowar green (27)	13.11±0.74	63.36±4.98	53.86±4.60	994.57±79.92	0.43±0.03	1.69±0.34	0.75±0.07
Neem leaves (2)	8.46±0.34	43.13±12.59	29.64±3.04	780.15±7.06	0.30±0.12	2.84±2.45	0.70±0.002

Figures in the parentheses indicate number of samples analysed.

Table 3: Mineral Content in Hair Samples of cows

Particular	Ca (%)	P (%)	Mg (%)	Cu (ppm)	Zn (ppm)	Mn (ppm)	Se (ppm)
Hair samples (n=15)	0.32±0.029	0.02±0.005	0.18±0.013	6.28±1.65	73.51±4.00	88.27±9.35	2.82±0.24

Table 4: Macro and Micro-Minerals Availability vis-à-vis Requirement for a cow (450 kg Body Weight) Yielding 10 kg Milk (6% Fat) PerDay

Attributes	DMI (kg/d)	Ca (g)	P (g)	S(g)	Cu (mg)	Zn (mg)	Co (mg)
Mineral requirement	11.50	64.5	42.80	23	115	920	5.75
Daily mineral availability from traditional feeding	11.50	50.65	29.15	21.35	75.07	333.63	3.56
% deficiency		21.47	31.89	7.17	34.72	63.73	38.08

Table 5: Mineral Requirements for Lactating cows

Particular	Cows	
	Calcium	Phosphorus
Maintenance (g)	18	13
Milk yield (g/kg Y)	4.65	2.98

Table 6: Specifications for Area Specific Mineral Mixture for Cuddalore District

S. No.	Characteristic	Requirement
1.	Moisture (%), Max.	5.0
2.	Calcium (%), Min.	22.0
3.	Phosphorus (%), Min.	14.0
4.	Sulphur (%), Min.	1.90
	Sulphur (%), Max.	2.75
5.	Copper (%), Min.	0.15
6.	Zinc (%), Min.	1.40
7.	Manganese (%), Min.	0.14
8.	Cobalt (%), Min.	0.013
9.	Iodine (%), Min.	0.026
10	Chromium (%), Min.	0.004
11	Fluorine (%), Max.	0.09
12	Acid insoluble ash (%), Max.	3.00
13	Lead (ppm), Max.	35
14	Arsenic (ppm), Max.	10

Note: The values for requirements (2) to (14) are on moisture-free basis.

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REVIEW ARTICLE

Physical Activity, Good Nutrients and Circadian Rhythmicity of Humans-Systematic Review

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ABSTRACT

Physical activity with good nutrition keeps our circadian rhythm in rhythmicity, keeping the body and mind healthy. In the rapidly developing 21st century, people are busy building their own and their loved ones' careers. When we are taking our brain and body for granted, it becomes essential for us to rethink what we are doing in return for them. Stress, pressurising one's body and mind to work for us, irrespective of day and night is what we do in return. Working at night has become very common but it is creating drastic effects on the human body. Circadian rhythms are the 24 cycles that have the ability to affect the physiology, behavioural patterns, emotions, and metabolism of the human body. Maintaining the circadian rhythm is very important because it has control over all the physiological activities of our body. Physical activity during the rest phase can affect the sleep cycle since melatonin hormone production is affected. Shift workers are forced to sit awake, eat and wake during the night, this can significantly bring down the functioning of the circadian rhythm. Imbalance in the circadian rhythmicity leads to several disorders in our body such as Type II diabetes, cancer especially colon cancer, breast cancer in women, obesity, emotional down, heart diseases, etc. Keeping in mind the alarming risk of imbalance in circadian rhythm in the present generation, the present article discusses interlinks between physical activity, circadian rhythm and sleep. The role of hormones in maintaining circadian rhythmicity. It also discusses how a timely and balanced diet maintains the circadian rhythm. Further studies are required to bring in the solutions for maintaining the circadian rhythm.

Keywords: Physical activity; Nutrients; Health; Circadian rhythm, Cardiovascular activity.

INTRODUCTION

Physical activity and good nutrients are very essential for maintaining a healthy lifestyle. These two factors alone cannot keep us healthy, because circadian rhythmicity is also going to be very important in maintaining physiology of human health. Circadian rhythm is where one's mind, body, and behaviour are in a 24hour cycle. However, sleep is also one of the factors which are interlinked with the circadian rhythm. Sleep in young people is disrupted for many reasons and may lead to anxiety and depression. Depressive symptoms are also seen when a person takes a long time to sleep (Nutt, D., Wilson, S., & Paterson, L. 2008). Acquiring proper nutritious eating, physical exercise and sleeping practices is the most important mediate to decrease the chance of any neuropsychiatric disorders (MatteoBrigulio et al, 2019). Some facts have pointed out that short sleep duration leads to greater awake time which is directly proportional to more energy intake, however, increase in energy intake without adequate sleep would lead to severe metabolic imbalance for a human being (Kristen L Knuston, 2021). A mechanism through which the physiological processes, such as meals and sleep, and the dark/light cycle can be coordinated, is developed in many organisms, including mammals (Takahashi, 2017). The evolutionarily conserved circadian rhythm integrates with external cues, the zeitgebers, to coordinate the light/dark cycle with physiology, metabolism, as well as behaviours (Cheng and Cheng, 2021). Though there is not much of a change in the light/dark cycle with evolution of the circadian clock, there has been a significant change in the zeitgebers that reset our clocks have changed significantly with time. Social and professional obligations force people to stay late at night. Light during the night and multiple other factors mislead the internal clocks amongst the shift workers whose sleeping and eating patterns are often at the wrong time (Roenneberg and Merrow, 2016). The resulting loss of synchrony can lead to serious consequences, which include sleep, cancer, psychiatric disorders, heart attacks as well as metabolic disorders (Lee S. et al., 2010; Scheer et al., 2009). Studies also showed that there is an interplay between redox biology and circadian rhythmicity. These two factors can synergistically confer protective health effects following regular exercise (Conor McClean et al., 2022). It is found to be reported that oxidative stress, a condition where the reactive oxygen species outcompete the level of antioxidants, can have an effect on circadian rhythm. Prior research also mentioned the oxidative stress induced by H_2O_2 administration caused phase shifts of the PERIOD2:LUCIFERASE bioluminescence rhythm in mouse embryonic fibroblasts invitro and in mouse peripheral tissues in vivo. (Yu Tahara et al., 2016). (Baba et al., 2015) reported that the hormone melatonin induces the PERIOD2: LUCIFERASE bioluminescence rhythm in the cornea of mice.(Baba et al., 2015). This article emphasises on the roles that nutrition, sleep, and physical activity play in synchronising the mammalian circadian clocks, and how desynchrony can affect human health.

Interlinks between the physical activity, circadian rhythm, and sleep

Circadian rhythms are the 24 cycles that have the ability to affect the physiology, behavioural patterns, emotions, and metabolism of the human body (Nasso et al., 2019). Suprachiasmatic Nucleus (SCN) of the hypothalamus in the human brain is the centre for the circadian rhythms, keeping this active keeps all the functions of the body and mind active (Nasso et al., 2019; Peptide et al., 2004).

Sleep resets the Circadian Rhythm

The most leading zeitgeber and a vital component in setting the sleep/wake cycle is the light/dark cycle, disruption of which can lead to the disturbance in the circadian rhythms, especially in case of jet lag, artificial light at night (LAN) and shift work, by clock gene expression alteration in intensity and duration-dependent manner (Shuboni and Yan, 2010). The cerebral cortex of mice showed alteration, inaccessibility of chromatin and the attenuation of expression of clock gene, this was reported for a minimal sleep disruption of even a single day (Hor et al., 2019). A recovery within 48h was observed to be phenotypical, however a disturbance in expression of gene continued for nearly 7 days, indicating the chronic effects of even short-term sleep disturbance. Sleep disruption can occur even on acute exposure to LAN. While phase shifts and attenuation of rhythms are possible even with short term exposure, chronic exposure increases the risk of fatal diseases like heart attack, stroke, and cancer, especially among night shift workers and flight attendants (Brown et al., 2009; Arendt, 2010; Lee S. et al., 2010). Shift work, a potential carcinogen, also elevates the risk of heart disease (Yousef et al., 2020; Schernhammer et al., 2001; Davis and Mirick, 2006; Megdal et

al., 2005; Vetter et al., 2016). Sleep disruption causes multiple clock genes to lose its rhythm (Archer et al., 2014). The disruption in expression of the clock gene is linked to a lesser prognosis of cancer, possibly due to loss in rhythmicity of the gene, period circadian regulator 2 (PER2) (Cadenas et al., 2014). Period circadian regulator 2 (PER2) has a role in tumour suppression, by p53 stabilisation, also through cell cycle regulation (Farshadi et al., 2020; Wood et al., 2008; Gotoh et al., 2014; Xiang et al., 2008). Hence when PER 2 is downregulated, there is an increase in the levels of Cyclin E and Cyclin D, which in turn speeds up tumour growth in case of breast cancer, disrupting DNA damage repair pathways (Yang et al., 2009; Fu et al., 2002; Xiang et al., 2008). Studies have reported that there was significant genomic hypo-methylation in people working in night shifts in comparison to shift workers working during the day time. This can possibly make us conclude that a disturbance in the internal circadian clock can lead to cancer through perturbation in the genome (Bhatti et al., 2015). Hence, LAN, chronic jet lag, and shift work can significantly cause an increase in cancer and various diseases through multiple mechanisms, which includes covalent modifications to DNA, down regulation of clock genes, and rhythmic expression attenuation. However, further studies are required to assess the link between cancer and shift work, because there is also contradicting evidence on how shift work does not participate significantly towards carcinogenesis (Yang et al., 2021). Sleep disruption can occur even due to heritable mutation, in addition to other environmental factors. This is observed in familial advanced sleep phase syndrome (FASPS), characterised by the sleep/wake cycle showing a significant shift (Jones et al., 1999). Many factors are responsible for disrupting both the quality and quantity of sleep. Hence, minimising sleep disturbance as much as possible for the betterment of the function of the circadian as well as improving the overall health, becomes very important. It has been reported that sleep deprivation among men and women leads to a decline in the level of sex hormones which again is connected with the circadian rhythm. Many reports suggest that this can be one of the reasons for infertility (Olubodun Michael Lateef et al., 2020).

Physical activity Brings change in the Circadian Rhythm

The phase shift that is induced by exercise is time-dependent, hence the time of the exercise has a vital role in regulation of the circadian clock. Exercise has a capacity in the shifts and resetting circadian rhythm. Studies have proved that night-time exercise has no effect which simply promotes night work and delays bedtime (Healy et al., 2021). But there are studies that have shown that the power output of ballistic training exercises such as squat jumps and bench press throws which are known to increase muscular power is not due to circadian variation in resistance-trained young men. (Disa L. Hatfield et al., 2016).Another study by ÖzgürEken et al., 2022 presented the effects of high-intensity functional exercise namely kickboxing among young athletes male of the age group between 18-25 on circadian rhythm using vertical jump, average power, anaerobic power, and T-line agility values as performance parameters. It has been noticed that, in healthy adult men, early evening exercise sessions (before melatonin onset) causes a significant phase advance, while late night exercise can cause a significant phase delay (Buxton et al., 2003). Forced acute exercise in mice resulted in their skeletal showing a stimulation of carbohydrate metabolism during the early rest phase. However, forced exercise in the early active phase showed an activation in HIF-1a signalling cascade as well as a stimulation in lipid metabolism, ketones, and amino acids (Sato et al., 2019). Though exercise is an important zeitgeber for few peripheral clocks, it is not as important as meal times for entertainment. Indeed, the phase shifts resulting from exercise can be dependent on meal times too (Sasaki et al., 2016b). Though physical activity comes next to feeding as a dominant zeitgeber, timely exercise cannot maintain the peripheral clocks if the meal time is out of phase. Therefore, exercises done late at night can have an impact on any human being, and not just the shift workers. Even then we have studies which say exercise can have a major circadian phase-shifting effect on humans, affecting their sleep and wake pattern. The exercise function is also influenced by the disruption of the circadian cycle. A mutation in the CLOCK circadian gene causes severe losses in muscular power and exercise endurance (Andrews JL et al, 2010). Hence further studies are required to check which zeitgeber has an effect on the circadian rhythm.

Food modifies circadian rhythm

Usually, the two cycles, namely, the light/dark and sleep/wake cycles are closely aligned to the feeding/fasting cycle. An untimely meal can result in a phase shift in the peripheral tissues, which in turn leads to deteriorating impact on overall health of human beings. Peripheral tissues-SCN asynchrony, has resulted in reduced tolerance towards

glucose, shift of phase in cortisol secretion, increased levels of triglycerides as well as elevated risk of obesity (Morris et al., 2016; Marcheva et al., 2010; Grant et al., 2021). Food composition and nutrients has effects on cortisol levels which inturn modifies the circadian rhythms and has these physiological changes, which cause a rise in obesity, heart diseases, diabetes, cancer, multiple age-linked diseases (Scheer et al., 2009; Davis and Mirick, 2006; Shi et al., 2013; Gan et al., 2015; Haus and Smolensky, 2013). Meals is the most important zeitgeber for the peripheral clocks. An untimely meal could result in a phase shift of 12 hr and more (Salgado-Delgado et al., 2013; Le Minh et al., 2001; Damiola et al., 2000; Sujino et al., 2012; Wehrens et al., 2017; Wolff and Esser, 2012). Eating during the night has resulted in attenuation of rhythms and a drop in overall sleep timing (Grant et al., 2021; Morris et al., 2016). Phase shifts in the peripheral clocks can result from a delay in just a few hours (Wehrens et al., 2017).

Intermittent fasting (IF), where a person restricts meals for about 6–12 h/day, is proven to have multiple advantages like anti-ageing, reduced risk of diabetes, and weight loss (Schenk et al., 2011). Studies on mice have proven that time-restricted feeding caused a decrease in fat accumulation, increased insulin sensitivity, reduced inflammation, and improved glucose tolerance (Chaix et al., 2014, 2019). Research conducted on humans showed that many of them restricted their meals to the afternoons and evening time, inturn delaying the time cues which otherwise reset the peripheral clocks in the morning. Instead of enhancing the overall health, late dinners could cause a rise in obesity (Tahara and Shibata, 2013). A shift in the timing of restricted feeding by starting it during the onset of the active phase would possibly enhance the possibility of receiving multiple benefits of fasting in the participants (Sutton et al., 2018), without disturbing the circadian clock (Regmi et al., 2021), however contradicting evidence do exist(Adafer et al., 2020). The circadian system, like physical activity, prepares the body for nourishment and encourages all through the day, the energy substrate is stored in specific tissue. Some dietary compounds can influence the circadian rhythm. Components such as alcohol disrupt the biomolecular, hormonal, and behavioural circadian rhythms in humans and other animals. Coffee in the evenings slows down the circadian system in vivo and also has capacity in entraining humans with chronic circadian rhythm (Potter et al, 2016). However, studies also say that careful use of caffeine impairs sleep following jetlag, work at night, etc (Beaumont M, Batejat D, Pierard C, et al., 2004). The nutritional value of the meals is as important as timely feeding. The Ketogenic diet, which is a high fat diet, has been effective in curing type 2 diabetes and epilepsy (Napoleão et al., 2021). Ketosis-inducing diets, though helpful in improving many syndromes, have shown to have a negative impact on the circadian clock (Stucchi et al., 2012; Kohsaka et al., 2007). Studies have shown that, obesity resulting from a high-fat diet increases the expression several important clock genes Dbp, CK1ε and Bmal1, in mouse livers as well as Clock, Bmal1, Per1/2/3, Dbp, CK1ε, and Cry1/2 in the kidneys (Hsieh et al., 2010).

Relation between hormones and Circadian Rhythm in Men, Women, and Children

Working at night increases melatonin hormone and also the reproductive hormones shoot up which may result in activating the cancer cells in women and may lead to breast cancer (Davis &Mirick, 2006). Evidence showed that the onset of puberty has an impact on the ultradian rhythm of hypertension and pulse rate (Gumarova et al., 2021). The mechanism of ultradian cardiac rhythm is not known and includes ethological patterns of physical activity, vasomotor and exertional fluctuations of the cardiac rhythm. Studies proved that the autonomic nervous system (ANS) acts as a thermostat in variation of circadian hypertension. ANS has a capacity in generating ultradian cardiac rhythms. This was given by (Hadtstein et al., 2004) when they observed children's rhythmicity was consistently higher during the day than in night times, while heartbeat and blood pressure did not show much variation. Men who work in shifts to varied professions like health practitioners, lawyers, and police have to work during night and have a high risk of prostate cancer (Davis &Mirick, 2006). Studies done among Caucasian Men and African American men showed that out of 553 men 5 of them were prone to either prostate cancer or colon cancer (Krstev et al., 1998). According to a recent study, individual circadian preferences (chronotype) differ by sex and age (59% women, 186,289 individuals, 164 research). Compared to women, men are, on average, generally evening orientated. (Cappadona et al., 2020) The distinctions between men and women, on the other hand, fade through time: young women and older women compared to older men and young men were morning-oriented and less morning-oriented, respectively (Randler et al.,2019). The increasing size of the body link negative health factors, which majorly suggests the evening chronotype. A group of researchers conducted a study to assess the links between

gender, chronotype, and several health-related features (Fabbian et al., 2016). Morning-type (MT), Intermediate-type (IT), and Evening-type (ET): are the most common chronotypes. The early half of the day is when MT reaches maximal activation, whereas the second half of the day is when ET reaches its peak. As a result, the terms 'lark' (early riser) and 'owl' (late sleeper) are commonly used to describe MT and ET persons. Although salivary cortisol range does not explain differences in wake-up time or sleep time length in MT (Kudielka et al.,2006). Individual preference has a genetic foundation for a specific time of day, and extensive genome-related research has uncovered many previously unknown genes (Cappadona et al., 2020). Rough data suggest that different chronotypes follow a Gaussian manner distribution (10% MT, 10% ET, 80% IT) (Ashkenazi et al., 1997). In terms of general health, most research on teens revealed a link between ET and harmful routines like lower physical activity, higher consumption of sugar, fats, and high smoking and alcohol habits. Links between teenagers (women and men) following late bedtime, late wake-up time, and ET, particularly on weekends were studied. When their energy intakes were compared and matched, women who consumed a larger ratio oftheir daily consumption later in the day were more likely to lose weight than those who devoured afterwards in the day (Potter et al, 2016). ET due to less sleep efficiency had a poor life quality. In terms of psychological aspects of health, various researchers have found a link between ET and impulsivity, anxiety disorders, depression, nightmares (particularly in younger women), alcohol, stimulant usage, and psychopathology (Fabbian et al., 2016). One of the psychopathological traits is risk-taking, a complex kind of decision-making that includes a calculated assessment of probable costs and rewards, whether immediate or postponed. Although males are more likely to take risks, ET ladies take much more trouble than females in IT and MT(Gowen et al., 2019). According to recent research, a group of women in the United States, ET was linked to an increased risk of cardiovascular health (OR 2.41), not achieving the AHA (American Heart Association for Women) diet (OR 2.89) and physical activity standards (OR 1.78), and shortening sleep (OR 2.15) (Makarem et al., 2020). Furthermore, when compared to MT, ET had a considerably higher risk of death (Knutson et al.,2018).

Many medical illnesses include symptoms causing nonfatal and fatal situations (Smolensky et al.,2015). The incidence of cardiovascular (CV) acute events, in particular, does not occur at random throughout the 24-hour periods but instead follows distinct temporal patterns as a result of cyclic fluctuation in pathophysiologic processes or environmental stimuli (Manfredini et al., 2013). Women's under-representation in cardiovascular research, on the other hand, remains a barrier to knowledge generation and the development of clinical practice guidelines (Norris et al., 2020). A time-related approach might assist in providing more effective patient treatment by understanding the primary symptoms of cardiovascular (acute) illnesses and initial endogenous processes. Chronotherapy, or therapy tailored to the time of day, accumulates a substantial body of evidence. Despite this, it is still on the periphery of clinical practice and drug development investigations (Ribeiro et al., 2021). On one side, customized chronotherapy functions on internal circadian time are unique to each person and imparted by sex, age, genetics, and environment. Therefore, attempts to gather biological markers based on blood type for individualised circadian is challenging. Attempts proposed to gather blood-based biomarkers for individualised circadian determination were reported (Laing et al., 2017). On the other side, proven evidence is required, with particular attention to variances in medication reactions, taking into account any sex and gender-specific disparities. Several bodies and gender-related characteristics influence medication reaction. Gender differences can also significantly impact medication safety profiles, with women having a higher response to the adverse drug than males (Mauro et al., 2019). For cardiovascular drugs, such as acetylsalicylic acid (ASA), which are generally dosed in the morning. When comparing morning with evening delivery, the evening group showed a substantial decrease in the aggregation of platelets. However, the ASA response in the morning varied across the sexes. Men's platelet reactivity reduced, whereas women's rose. Women may benefit more from switching ASA from morning to evening (Krasinska et al., 2019). The aspirin doses given to pregnant women in the morning and evening to avoid high blood pressure, on the other hand, showed no change (Shanmugalingam et al., 2019).Hormonal homeostasis fluctuates on a regular basis; it's now evident that hormones and circadian cycles are intricately linked, and that the internal clock connects with the external factors to keep the body in balance. Hormones like growth hormones are among the hormones that have been demonstrated to undergo daily oscillations also are the nutrient-sensitive hormones, have an association with circadian rhythm, and their release is influenced by environmental factors like meal time and light–dark cycles, at

least in part(Davide Gnocchi and GiovannellaBruscalpi, 2017). Melatonin serves a variety of functions and can be thought of as the "relayer" that relays information about light–dark cycles. There are also endogenous circadian systems that regulate glucose metabolism and analogous lipid metabolism cycles, which are regulated by the actions of numerous clock genes. Obesity, insulin sensitivity, diabetes, hormonal imbalance, and hunger dysregulation are all linked to sleep disruption, which has a deleterious impact on hormonal cycles and metabolism. In mammals, melatonin has a role in the control of reproductive behaviour and sleep. When the sleep cycle and the intrinsic circadian clock are out of sync, adverse health repercussions such as hormonal or metabolic disorders arise(Kim et al, 2015).

CONCLUSION

Physical Activity, Good Nutrients and Circadian Rhythmicity in Humans are the most important factors of human life. Physical activity is a must in our life, but when we should indulge ourselves in physical activity plays a vital role. Daylight keeps our circadian rhythm active in this duration we should involve ourselves inactivity so the correct utilisation of energy could take place. Good food intake here plays an important role because energy utilisation happens when the metabolism of food takes place. The composition of the food controls the circadian rhythm and also at the right time. Right time means in the daytime, taking food during night attenuation of rhythms. Hormonal changes like the production of a high concentration of melatonin during night-time if we are performing any kind of physical activity, creates fatigue in the muscles. Melatonin is the relaxing hormone during its production body should be at rest. Since shift workers, lawyers, medical practitioners keep themselves awake and eat during night, it becomes very common but may result in colon cancer, development of tumours, reduction in insulin production resulting in type II diabetes, Obesity etc.

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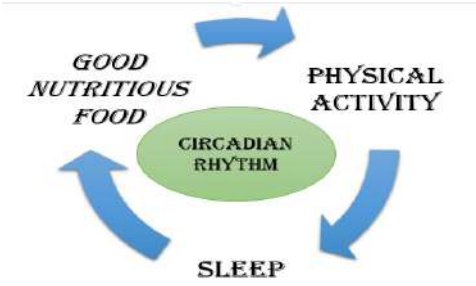

Figure 1: Circadian Rhythm showing its relation with Food, Sleep and physical activity

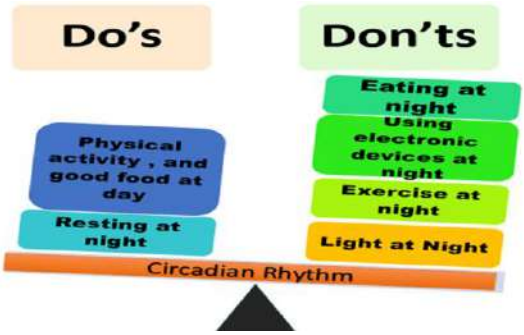

Figure 2: Factors causing imbalance in the circadian rhythm

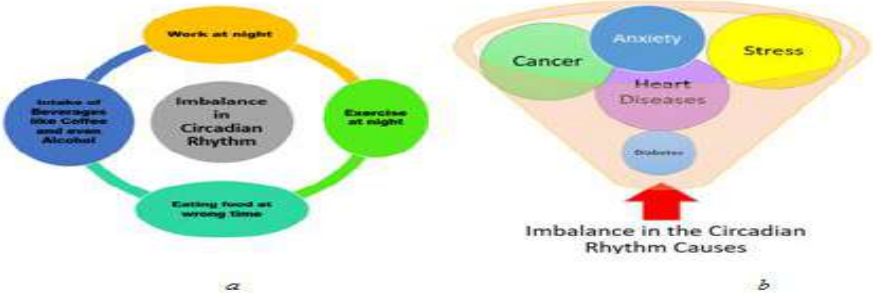

Figure 3: a) Factors affecting circadian rhythm b) Imbalance in the circadian rhythm causes

RESEARCH ARTICLE

A Hybrid Deep Learning Technique for Predicting Adverse Drug Reactions

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ABSTRACT

In order to make the drug development pipelines more potent and effective, as well as to reduce their influence on lives of the patients, the identification of probable ADRs at initial stage is necessary. Even though the current prediction strategies have attained optimistic results, they are unable to find enough details other than the chance of ADRs occurrence, or they need comprehensive drug information which is frequently unobtainable for ADR prediction. This research work focuses on the deep learning methods which utilize the drug profiles to make predictions and to exploit multiple attributes derived through several sources of data. In the initial stage, the missing features will be removed using min-max method in the data preprocessing stage itself. Further, a novel system of deep neural network is established in this research work. This hybrid framework termed Hybrid Deep Neural Network (HDNN) incorporates a deep neural network based fuzzy inference system. The deep neural network and the fuzzy inference approach both have their individual proficiency in this HDNN model and because of these collective features, this model has been employed in this proposed work. In addition, to determine the optimal values for learning rate and momentum coefficient, a dominant optimization tool named Improved Binary Bat algorithm (IBBA) has been proposed. The experimental results reveal that the proposed approach delivers the optimum prediction rate of ADR when ingesting particular drugs and also recommends the guidelines at the process of drug development.

Keywords: Adverse Drug Reactions (ADR), Hybrid Deep Neural Network (HDNN), fuzzy inference system, Improved Binary Bat algorithm (IBBA), prediction.

INTRODUCTION

Drug safety and the post marketing surveillance is a key topic due to the exceptional rise of toxicology and clinical safety failures. Adverse drug Reactions are the effects caused by consumption of a drug for standard use at a regular/prolonged dose or by the combination of two or more drugs [1].The study reveals that the morality rate and reported serious have been increased from 5,519 to 15,107 and 34,966 to 89,842 respectively during 1995-2005. The World Health organization (WHO) encourages the research carried on ADRs due to some vital reasons: ADRs are one of the significant reasons for the serious effects and deaths in all countries. Even though the most of the ADRs can be avoided, no drug proves to be risk free [2]. An Adverse Drug Reactions (ADR) is defined as the harmful event that occurs due to the consumption of drugs. ADRs encompass the risks due to some therapeutic treatments or modification of the drug dosage or sudden termination of the drug. Even though every newly developed drug has undergone preclinical analysis and various stages of clinical experiments in the premarket observation, yet there is a chance of ADRs occurrence, since the drug is experimented clinically with not less than a few thousand patients and has numerous restrictions, like the short term clinical experiments or neglecting the patients who attain other medication or the aged persons [4]. Being the most significant issue in healthcare, the ADRs have been identified and predicted in the post market surveillance to get rid of the drawbacks of the premarket surveillance, as they have necessitated in pharmacovigilance also termed as drug safety surveillance. The occurrence and seriousness of ADRs may differ due to the drug features viz., the bioavailability, drug type, dosage, treatment duration, and administration route, and characteristics of the patient viz., age, sex, ethnicity, coexisting disorder, genetic, or geographic factors [5]. The frequency of occurrence is more among the aged persons; however the age is not the sole reason. Most of the ADRs are related to dosage as well as an individual's allergic reactions/idiosyncratic. The dosage related ADRS are predictable whereas the non-dosage related ADRs are unpredictable.

In general, the Adverse Drug Reactions (ADRs) are categorized into mild, moderate, severe or harmful. The severe or fatal ADRs described in the physician's prescription are precisely mentioned by the drug manufacturers as black box warnings. The consumer or the patient may observe the symptoms and signs immediately after the initial dose or may be known only after the prolonged use[6] The common outcome of mild ADRs in the elderly person is due to the functional deterioration, deviations in psychological state, lack of appetite, uncertainty, and dejection. The symptoms which are observed after consumption of drugs are correlated to the usage of drugs. Diagnosing symptoms that result from prolonged consumption of drug is complex and requires a major level of suspicion. Sometimes stopping of the drug is needed, but it is difficult due to the fact that the essential drug does not have an acceptable replacement. In the cases of severe allergic reactions due to the drug consumption and its signs, rechallenge can be done [7]. The Med Watch alert system (an ADR monitoring platform founded by the Food and Drug Administration) is the only source to recognize and scrutinize the sudden ADRs that receives the reports directly from physicians in the occurrence of suspicious adverse drug reactions. To reduce the dose-related adverse drug reactions, reducing the dosage of the drug or eliminating the causing factors is sufficient. Complete dismissal of drug consumption is advised in rare cases only. The drug once discontinued it is not advisable to attempt again in case of allergy or idiosyncratic ADRs. Changing to a different type of drug is commonly advised forallergic reactions and for dose-related ADRs too [8]. Adverse Drug Reactions can be prevented based on the knowledge of the drugs and their potential reactions. Computer based examinations has to be used to identify potential drug interactions, when there is a modification or addition of drugs. When it comes to aged persons the drugs as well as the initial dosage must be chosen carefully. It is highly advisable to consider ADRs before commencing the symptomatic treatment, in case of non-symptomatic patients.

Different computational methods varying from conventional statistics methods to advanced methods can be applied to solve the problems in the field of machine learning and data mining [9]. Machine learning and data mining are associated fields. Machine learning is derived from the field of Artificial Intelligence (AI) that aims the computers to learn like a human. This aim is precisely understood by examining the datasets, especially the complex data. Data mining has an applied perquisite of examining huge and complex datasets to find out new, invisible, yet valuable

knowledge in data [10]. Though Machine learning and data mining has its own goals and interests, they are fairly connected to statistics and share common methods. Deep Learning avoids complicated feature engineering, because the neural network itself creates the features automatically during the learning phase. A Deep Neural Network (DNN) system is known to be an Artificial Neural Network (ANN) with several layers between the input and output layers. In spite of the data being linear or non-linear, the DNN transforms the input into output by finding the optimized mathematical operations [11]. The DNNs are named as deep networks because complex DNNs has many layers with mathematical manipulation at each layer. The remaining part of this research paper is organized as follows: Section 2deals with some associated study regarding Machine Learning and Deep Learning for ADR predictions; Section 3 provides detailed description on the proposed methodology, the details of execution as well as the empirical findings are provided in Section 4.Ultimately, Section 5 confers the conclusion.

MATERIALS AND METHODS

This research work presents a computational framework called Hybrid Deep Neural Network (HDNN), known for its ultimate accuracy in the prediction of ADR. The missing features will be eliminated by the min-max method during the data preprocessing stage. This study results with a futuristic new design of the deep neural network. In this hybrid model a deep neural network is incorporated with a fuzzy inference system. The deep neural network, as well as the fuzzy inference system, has its own unique abilities and that is the main reason of using this model in this study and design. An Improved Binary Bat algorithm (IBBA) has been recommended as a dominant optimization tool to discover the optimum quantity of Membership Functions(MF) for each input along with the appropriate values for learning rate and momentum coefficient. Figure.1 represents the complete process of the prediction of ADR by using HDNN model.

Data Representation

In this research work, two types of drug information were utilised to define drugs from the biological and chemical perception, together with their chemical structures and correlated protein targets [12]. That is, three types of data features were used for defining the drugs. The Fig.2. illustrated the depiction of drug data, where n1 , n2 are the numbers of different types of features, and m being the total number of side effects considered for prediction. The well-known public drug information database Drug Bank is used in this study to retrieve the chemical structures of the FDA –approved small-molecule drugs and map them to PubChem database using the compound ID. In translating the data associated to the substructures of the drugs, SMILES (Simplified Molecular Input Line Entry Specification) is used in this study. These substructures were defined by having the segment rules (acquired using the chemical toolbox, Open Babel) as base is changed to PF2 format (accompanies the integers 0 to 1020 to encrypt variety of structures). The chemical substructures which are explained in PubChem will be encrypted as binary features (i.e., $c1$ _ $cn1$ in Fig. 2) as follows: in case the respective PubChem substructure is present in the drug, the entry was 1; if not the entry was 0. The protein data retrieved for each drug were collected from the Drug Bank using the UniProt ID. The proteins that are collected fron the Drug Bank are mapped to the UniProt Knowledgebase that contains the overall details about the proteins. Similar to the binary feature representation used for the chemical substructures, proteins (target) were converted as binary features for every drug to specify the existence or nonexistence of the respective proteins. In addition to the chemical and biological perspectives, the data on side effects is extracted from the SIDER database. This possesses the data regarding the medicines in the market and associated adverse drug reactions [12]. To ensure the reliability with the other drug related data, SIDER utilizes STITCH compound identifiers that were mapped into PubChem compound identifiers. To identify and predict its existence each side effect associated with the data for each drug can be classified as a binary target class (i.e., either as positive or negative)

Data Normalization

Data Normalization is the preprocessing technique used to transform the data into a refined format. The success of the Machine Learning algorithm relies on the quality of the datasets chosen. Data Normalization is a significant transformation technique that increases the accuracy and achieves better performance for the chosen data sets. Recognizing the importance of transformation technique in data mining algorithms, normalization technique is utilized in the proposed work to increase the generalization process and learning capability with minimum error. Generally, the attribute or feature values in the dataset are of different kinds viz., integers or decimal values. For managing and organizing, the feature values are scaled to a specified range in the dataset as a data normalization technique. Normalization is used in classification and clustering techniques since the input data points should not be swamped in terms of distance metric. This reduces bias and speeds up the training time in the classification process, since each feature value starts in the same range.

Min-Max Normalization

By using linear transformation, the min-max normalization method normalizes the dataset and converts the input data into a new fixed range [12]. The association between the original input value and the scaled value is maintained by min-max method. When the normalized values diverge from the primary data range, an out of bound error is encountered. This method is used to confirm the extreme input values are within a specific range. Min-max normalization converts a value X_0 to X_n which fits in the specified range is illustrated by the equation (1)

$$X_n = \frac{X_0 - X_{min}}{X_{max} - X_{min}} \qquad (1)$$

Here, a new value for the variable X is represented as X_n,and the existing value for variable X is represented X_0,besides the minimum data point in the dataset is signified by $X_{min,}$ and the maximum data point in the dataset is notated by $X_{max.}$

ADR prediction using Hybrid Deep Learning Model

A Hybrid Deep Neural Network (HDNN) achieves better performance in classification even without using the feature extraction phase compared to other machine learning approaches. The following section narrates the concept of ADR prediction using Deep Learning with fuzzy inference system.

Deep Learning

Deep Learning is an efficient and effective machine learning approach to attain the predictions from complex data sources in a most accurate manner. It has the capability of learning the features on their own; conversely they depend on predefined structures and labeled data. The next problem to deal with Deep learning methods is to interpret and extract the features that are learned by the networks. A classification label is not sufficient for certain applications, in such situations understanding the high level features that emerges from the networks is equally important. The features generated in this manner are significant for two reasons. i)The features produced in the unsupervised system exempts the system burden by reducing the hand constructed labels. The problem in hand labeling data have frequently delayed the improvement of DL strategies as developing thousands of training samples is a challenging task;ii) It possesses the capability to identify particular neurons in the system of DL that suits with the appropriate feature which enables the analyst to get into the network and extract the feature arrays. The Deep Neural Network (DNN) is an Artificial Neural Network (ANN) that consists of multiple hidden layers between the input and output layers. The multiple hidden layers in the network provide optimal accuracy in the result, but the raise in the quantity of hidden layers gradually increases the complicacy of the system and hence the efficiency of training is decreased. So fixing the number of hidden layers is required during the training process. In this research work, aDNN is integrated with Fuzzy Inference System (FIS). The deep learning will extract the appropriate features from the raw data and these extracted features are fed to fuzzy inference system. This FIS is based on the Fuzzy Neural Networks (FNN) with multiple hidden layers. A FNN is a learning prototype that applies the metrics of the fuzzy systems by using the methods from neural networks. [13].

Integration with Fuzzy Inference system

The Fuzzy Interference System (FIS) will process the features that are extracted from the data via DL. This allows the system to build a human reasoning model and provides the mechanism for biasing the system through the feedback received from the analyst. This necessitates the deep learning algorithms to incorporate the Fuzzy logic via the extension principle. The inclusion of fuzzy logic integrates both the granularity of the data as well as the methods to handle the incomplete information received from each modality. These mechanisms will provide a method for individual evaluation of the value obtained from each modality. Among many FIS models, the Sugeno fuzzy model is the most widely used model due to its high interpretability, computational efficiency, built-in optimal and adaptive techniques [14].Fig. 3 illustrates the process of fuzzy inference system. The structure of the Fuzzy Inference System (FIS) consists of five functional blocks namely, Rule Base : containing the number of fuzzy IF-THEN rules, the Database that defines the membership functions of fuzzy sets used in fuzzy rules, the Decision-making unit performs operation on rules, Fuzzification Interface that calculate the fuzzy input, Defuzzification Interface to calculate the actual output. Fuzzy interference system uses the if-then rules which allows for complex non-linear problems approximation. The advantage of structured rule based systems is that it can be biased by subjective information. This gives a chance for an analyst to give the expert information to the system, thereby improving the outcomes of classification or changing the behavior of system [15]. The advantage of using this feedback is to speed up the learning of the independent systems during changing or uncertain situations. For instance, the FIS has three input(x,y,z) and the momentum algorithm is used as the learning rule and the purelin transfer function is used as the output function. suppose that the rules have three fuzzy *if-then* rules of Takagi and Sugeno's type.

Rule 1: If x is A_1, y is B_1, and z is C_1 then $f_1=p_1x+q_1y+t_1z+ r_1$,
Rule 2: If x is A_2, y is B_2 and z is C_2 then $f_2=p_2x+q_2y+t_2z+ r_2$,
Rule 3: If x is A_3, y is B_3 and z is C_3 then $f_3=p_3x+q_3y+t_3z+ r_3$,
Fig. 4 describes the process of Sugeno model FIS and the function of each layer is explained as follows:
Layer 1 Fuzzification Layer: Every node in this layer is an activation node. Include these nodes with a node function as

$$O_{1,i} = \mu_{Ai}(x), \ for\ i = 1,2 \quad (2)$$
$$O_{1,i} = \mu_{Bi-2}(y), \ for\ i = 3,4 \quad (3)$$
$$O_{1,i} = \mu_{ci-4}(z), \ for\ i = 5,6 \quad (4)$$

where $O_{1,i}$ is the membership grade of a fuzzy set $A = (A_1, A_2, B_1, B_2\ or\ C_1, C_2)$and $\mu_{Ai}(x)$, $\mu_{Bi}(x)$and $\mu_{ci}(x)$ are any suitable parameterized MFs and it shows the level in which the provided input x (y or z) satisfied the quantifier.

Further, the membership function for A can be the bell shaped parameterized MF:

$$\mu_A(x) = 1/(1 + |(x - c_i)/a_i|^{2b} \quad (5)$$

Where $\{a_i, b, c_i\}$ is the parameter(s) set for the member function and these parameters are referred as Premise Parameters.

Layer 2 In this layer, every node is a fixed node that its output is the product of all received signals.
$$O_{2,i} = \mu_{Ai}(x)\mu_{Bi}(y)\mu_{ci}(z), i = 1,2,3 \quad (6)$$

Everynode output stands for the Firing Strength of a rule.

Layer 3 Contains the fixed node labeled N of normalization:
$$O_{3,i} = \frac{w_i}{w_1+w_2+w_3}, , i = 1,2,3 \quad (7)$$

The outcomes of this layer are called as Normalized Firing Strengths.

Layer 4 Contains the adaptive nodes:

$$O_{4,i} = \overline{w}_i f_i = \overline{w}_i(p_i x + q_i y + t_i z + r_i) \quad (8)$$

As each node in this layer is product of the Normalized Firing Strength from the third layer and output of DNN, hence it is termed as Consequent Parameters.

Layer 5 Contains a single fixed node labeled ∑ with task of summation that estimates the complete output of DNN network as the summing up of overall receiving signals.

$$Overall\ output\ O_{5,i} = \sum_i \overline{w}_i f_i = \frac{\sum_i w_i f_i}{\sum_i w_i} \quad (9)$$

The goal of using the DNN is to decide the parameter of the fuzzy MFs which is the most difficult task in fuzzy modeling. Here, most of the parameters are chosen based on users' experiences and/or trial and error. Hence to automate this process, any of the computational technique is used to reduce the error. Hence, this study tend to utilize the HDNN to construct a fuzzy inference system (FIS) where its MF's parameters are modified by a Back Propagation algorithm that permits the fuzzy system to capture the spatial relationships between the data and finally, the outcomes are estimated efficiently. From Fig. 3, it is clear that still there are some parameters which persist non-optimal due to the usage of DNN. Factually DNN tries to enhance the parameters of FL whereas it may be captured within local minima. There are some parameters left over in both DNN and FL which will affect the performance indirectly, even though the DNN has a highly effective learning algorithm and will help the FL to find the suitable parameters. To solve this issue, an efficient optimization tool namely Improved Binary Bat algorithm (IBBA) is utilized for obtaining the best number of MFs for each input and the optimal values for learning rate and momentum coefficient. The detailed explanation of IBBA amalgamation has given subsequently.

Improved Binary Bat Algorithm (IBBA)

The BAT algorithm was developed based on the echolocation behavior of the bats. The bats will locate their prey by reducing the loudness and increasing the frequency of the emitted ultrasonic sound [16]. The Bat Algorithm has been developed based on the characteristics of the real bats. The mathematical expression of the fundamental process of BA is as follows. Each bat in this BA possesses three vectors, viz., a frequency vector, a velocity vector, and a position vector which have been upgraded at time step t as (10), (11), and (12):

$$V_i(t+1) = V_i(t) + (X_i(t) - Gbest)F_i \quad (10)$$
$$X_i(t+1) = X_i(t) + V_i(t+1), \quad (11)$$

in which,$Gbest$ stands for the best position and $F_i$stands for the frequency of ith bat which is revised as follows:

$$F_i = F_{min} + (F_{max} - F_{min})\beta, \quad (12)$$

Whereβ in the range of [0, 1] is a random vector drawn from a steady distribution. From the equation (10) and (12), it is clear that various frequencies promote the ability of exploration of the bats to the optimal solution. To a certain extent, these equations can assure the exploitation ability of the BA. A random walk function has been employed to increase the performance as follows:

$$X_{new} = X_{old} + \varepsilon At, \quad (13)$$

Here, X_{old} indicates a single solution that is arbitrarily chosen from the available optimal solutions, εstands for an arbitrarily chosen number within the range of −1, and 1, and the average loudness of all bats at this time stepis

signified by A. Make a note that rand is a random number equally scattered in the range of 0 and 1. The pulse emission rate (r) and loudness (A) controls the balancing between the global and local search. It has been recognized that as the loudness increases, artificial bats incline to perform diversification instead of intensification. The aforementioned parameters is modified as,

$$A_i(t+1) = \alpha A_i(t) \quad (14)$$
$$r_i(t+1) = r_i(0)[1 - \exp(-\gamma t)], \quad (15)$$

Here,α and γbeing constants,αrepresents the same meaning of the cooling factor. The first and second item of the equation influences the algorithm to perform global and local search respectively. To clear up optimization issues with binary search space it is recommended to use the binary bat algorithm (BBA). The structure of BBA is similar as the original BA in which the velocity and frequency has been designated in continuous space. BBA modifies original BA in two different ways:

(i)The vector of position is not a constant valued vector anymore but a bit string.
(ii) The arbitrary function expressed in equation (13) is inappropriate to binary search space anymore. Alternatively, a modest function has been adopted.

The position update equation for BBA transforms to

$$x_i^k(t+1) = \begin{cases} (x_i^k(t))^{-1} & rand \le f\left(v_i^k(t+1)\right) \\ x_i^k(t) & rand > f\left(v_i^k(t+1)\right) \end{cases} \quad (16)$$

Here,

$$f\left(v_i^k(t)\right) = \left|\frac{2}{\pi}\arctan\left(\frac{\pi}{2}v_i^k(t)\right)\right| \quad (17)$$

$x_i^k(t)$and$v_i^k(t)$defines the position and velocity of ith artificial bat at iteration$t$ in kth dimension and $(x_i^k(t))^{-1}$indicates the complement of$x_i^k(t)$.

The function expressed in equation (13) for BBA transforms to

$$X_{new} = X_{old} \quad (18)$$

Inertia weight strategies

Here,X_{old} still means a solution chosen arbitrarily from the existing optimum solutions. Hence, inertia weight approach plays a vital role in progress of sustaining the balance amid the processes of global search and local search. The contribution proportion of old velocity to its new velocity at the current time step has evaluated by the inertia weight approach that observes the search situation and the inertia weight value is modified based on one or more feedback parameters. When the velocity update equation (10) is examined, it is evident that this equation has two parts. The first item ($V_i(t)$) denotes the velocity of population and the second item ($((t) - Gbest)$) commands the velocity of the ith position ((t)) with guidance of the global best solution ($Gbest$). Apply the guidelines of the neighbor bat (kth solution) to produce optimum solutions. Due to this, the following velocity update equation of original BBA has been applied to adjust:

$$V_i(t+1) = \omega\big(V_i(t)\big) + (X_i(t) - Gbest)F_i\delta_1 + (X_i(t) - X_k(t))F_i\delta_2 \quad (19)$$
$$\delta_1 + \delta_2 = 1 \quad (20)$$

Here, inertia weight factor has been indicated by w that balances local and global search intensity of the ith solution by handling the value of old velocity (t), X_k indicates a solution better than many other solutions, which is arbitrarily picked from the population ($i \neq k$), δ_1 is self-adaptive learning factor of global best solution ($Gbest$) within a range of 0 and 1, and hence, a learning factor of kth solutionis denoted by δ_2 within a range of 1 and 0. The algorithm enabled to efficiently avoid local minima, since the kth solution information is utilized to conduct the ith solution. Due to the increment ofδ_1, the effect of the global best solution ($Gbest$) turns out to be greater than the kth neighbor solution (X_k), given as

$$\delta_1 = 1 + (\delta_{init} - 1)\left(\frac{iter_{max} - iter}{iter_{max}}\right)^n, \quad (19)$$

Here, the initial impact factor of $\delta_1$is indicated by δ_{init}, the maximum number of iterations is denoted by $iter_{max}$, where the iter represents the existing number of iterations, and a nonlinear modulation index is notated by n. I fiter increases, the δ_1 increases from δ_{init} to 1 nonlinearly, whereas δ_2 decreases from $(1 - \delta_{init})$ to 0 accordingly. With a small δ_1 and a large δ_2, bats are permitted to fly across the search space, alternatively flying toward. Conversely, a large δ_1 and a small $\delta_2$enable the bats to converge to the global best solution during the subsequent phases of the search process. Hence, the recommended approach is able to efficiently regulate the global search and enhance the convergence to the global best solution during the final stage of the optimization.

The magnitude of the velocity has been significantly handled by the inertia weight method, which is expressed by,

$$w = w_{max} * \exp\left(-m * \left(\frac{iter}{iter_{max}}\right)^m\right), \quad (20)$$

Here, the total number of iterations is denoted by $iter_{max}$, the current number of iterations is indicated by iter, besides w_{max} denotes the maximal inertia values, and $m$indicates constant larger than1. The diffusion of the solutions into binary search space is considered as further advantage of the recommended Improved Binary Bat Algorithm (IBBA), besides the results being more accurate.

Algorithm 1: Pseudo code of HDNN

Input: pre-processed data
Output: ADR prediction
Step 1: Input $x = (x_1, x_2, x_3, \ldots . x_n)$ denotes data matrix of n samples, $y = (y_1, y_2, y_3, \ldots . y_n)^T$as their corresponding output labels.
Step 2: Propagate activity forward: for layer i = 1, 2,…., k.

$$O_{1,i} = \mu_{Ai}(x), \quad for\ i = 1,2$$
$$O_{1,i} = \mu_{Bi-2}(y), \quad for\ i = 3,4$$
$$O_{1,i} = \mu_{ci-4}(z), \quad for\ i = 5,6$$

Step 3: Membership function for A can be any appropriate parameterized MF using the fuzzy inference system,
$\mu_A(x) = 1/(1 + |(x - c_i)/a_i|^{2b}$
Step 4: Update the hidden layers
$O_i = \overline{w}_i f_i = \overline{w}_i(p_i x + q_i y + t_i z + r_i)$
Step 5: Calculate the error in the output layer using the improved inertia weight based binary bat algorithm
$w = w_{max} * \exp\left(-m * \left(\frac{\delta}{\delta_{max}}\right)^m\right)$,

Step 6: Update the weight and biases of each hidden layer

$\Delta w_i = \vec{\delta}_j . O_{i-1}$

$\Delta \vec{b}_i = \vec{\delta}$

Step 7: Computes the overall output of DNN network as the summation of all the incoming signals.

$Overall\ output\ O_{5,i} = \sum_i \overline{w}_i f_i = \frac{\sum_i w_i f_i}{\sum_i w_i}$

RESULTS AND DISCUSSION

To examine the effectiveness of the recommended drug side effect prediction method, the datasets have been collected and utilized. The datasets comprises the FDA-approved small-molecule drugs collected from Drug Bank that accompanies the chemical substructures described in PubChem and associated drug side effects retrieved from SIDER. The suggested method has been authenticated through comprehensive experiment which is included in this dataset that has equipped in the first series of experiment; subsequently the in-depth analyses have been made to evaluate the suggested method from several aspects. Prior to begin the experiments, the dataset equipped for the first series of trials were concisely examined, in which 1002 individual drugs and 3903 side effects have been taken into consideration.

Evaluation Metrics

During the trials, several measures have been used that are often utilized in binary classification to examine the usage of the various approaches in the prediction of side effect. The estimated true positive (TP), false positive(FP), true negative (TN), and false negative (FN) rates are utilized to evaluate several parameters for the performance, such as precision, recall, F-measure, and accuracy. The precision is described as the ratio of retrieved instances that were relevant, whereas the recall is explained as the ratio of relevant instances that were retrieved. Even though it is often contrasting in nature, the processes of precision and recall are both significant in assessing the performance of a prediction method. Hence, these two parameters can be merged with equal weights to get a single metric, namely F-measure. The final parameter is called accuracy that has been described as the proportion of appropriately predicted instances associated to each predicted instance. Figure 4.illustrates the overall comparison of the proposed and existing Adverse Drug Reaction techniques (ADRs). From the results it concludes that the proposed Hybrid Deep Neural Network technique (HDNN) provides the high accuracy results of 98.46% whereas the PCA+ similarity method provides 88.2% and RF classification method provides 82.1%.

CONCLUSION

This research work has proposed a computational system that incorporates the Hybrid Deep Neural network to identify the ADR. Initially the missing features will be eliminated using min-max method in the data preprocessing stage itself. This HDNN model works on fusing a deep neural network with a fuzzy inference system. The deep neural network and the fuzzy inference strategy both have their individual proficiency in this HDNN model. To determine the optimal values for learning rate and momentum coefficient, an Improved Binary Bat algorithm (IBBA) has been proposed in this work that acts as a dominant optimization tool. On the whole, the empirical findings depict the promising classification performance and suggest the suitability of the represented approach for possible identification of ADR. This study can be further stretched to get exploited as an efficient tool in pharmacovigilance or can be amalgamated to existing approaches, specifically in the Food and Drug Administration's Adverse Event Reporting System or e-health records, concerning the enablement of drug safety through selecting the candidates that must be able to interact with the human body.

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Table 1. Comparison results of the existing and proposedADR prediction techniques

Metrics	RF classification	PCA + Similarity	HDNN
Precision	0.821	0.862	0.975
Recall	0.813	0.872	0.9754
F-measure	0.842	0.894	0.982
Accuracy	0.821	0.882	0.984

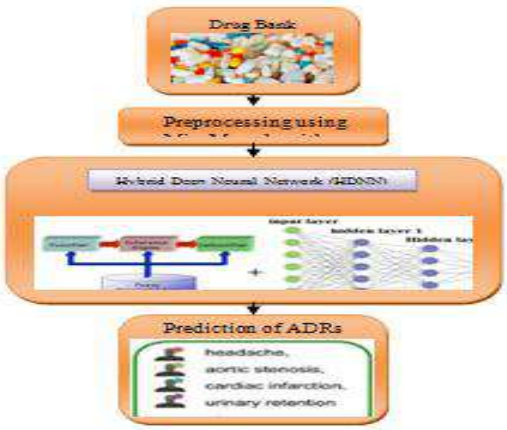

Fig.1: The overall process of the prediction of ADR using HDNN model

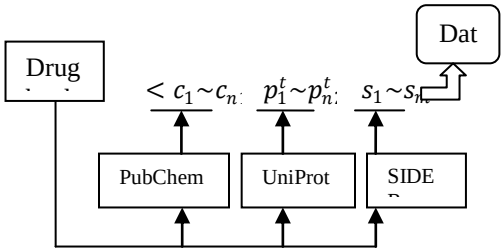

Fig.2. The overall data representation of drug data

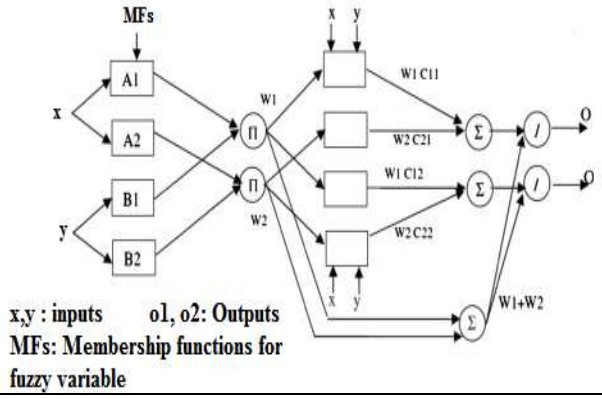

Figure.3. Process of Sugeno type Fuzzy Inference System

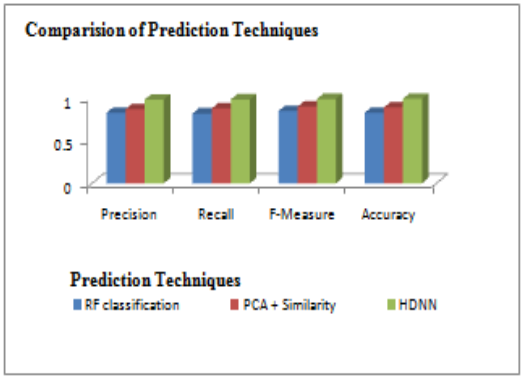

Figure 4. The Overall comparison of the existing and proposed ADR prediction techniques

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RESEARCH ARTICLE

Cyber Security and Artificial Intelligence in Social Media

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ABSTRACT

Over a billion people are connected through social media platforms like Face book and Twitter, which also allow them to converse and share information between groups of people. These social media platforms have the potential to greatly benefit society by disseminating knowledge about contagious diseases and discussing solutions to issues like eliminating child trafficking and violence against women. Social media platforms can harm individuals by sharing incorrect information, or "fake news," as well as by compromising their privacy. Human usage of social media platforms is evolving as a result of the widespread use of artificial intelligence (AI) systems, advanced machine learning techniques, and cyber attacks on information systems. The importance of AI and cyber security for social media systems is covered in this essay, along with the advantages of AI and how to safeguard social media systems.

Keywords: Artificial intelligence, Social media, , cyber security, Privacy

INTRODUCTION

Face book and Twitter are two examples of social media platforms that are being used for humanity's benefit. These systems, for instance, can give users with information on the spread of diseases, promote emergency planning, and enable information sharing so that users are better educated about a variety of topics, such as politics and sports. On these systems, a number of analytics tools are being used to extract data about users' posts as well as the users themselves. Although the nuggets that are recovered might benefit humanity, they might also jeopardise people's privacy. Additionally, social media platforms have been used as a means of disseminating untrue rumours that have the potential to injure people significantly. Additionally, cyber-attacks could affect the social media platforms and the analysis tools, which would compromise the submitted information. In addition to examining how social media

platforms could be safeguarded against cyber attacks, this article investigates the usage of AI approaches for social media. Additionally, the potential invasions of privacy are also discussed.[1] provides a thorough explanation on evaluating and securing social media. This research aims to analyze some of the new problems we are dealing with as a result of the spread of fake news and cutting-edge cyber attacks on social media platforms. This essay has the following structure. The artificial intelligence for social media. We'll talk about using AI to solve the difficult problem of identifying fake news, as well as using machine learning techniques to extract important information and aid humans. For social media networks, safety and privacy are covered . We'll talk about things like social media access management and potential privacy concerns brought on by social media data analysis. The integration of AI and security for social media. One possible attack would be against the machine learning methods used to analyse social media data. Therefore, approaches for handling such attacks using adversarial machine learning will be covered. It also includes a discussion of the work's future directions, the paper is finally summarised.

AI In Social Media

For several social media applications, like Twitter and Face book, machine learning techniques have been used. For instance, the user's location can be predicted, sentiment analysis can be done, and recommendations can be made using machine learning techniques. The InXite system, which offers many analytics capabilities, is reviewed in some detail [2].The most influential users of a social media network can be identified using machine learning techniques, which can also be used to forecast where the disease will spread next. To manage emergency operations during earthquakes, hurricanes, tornadoes, acts of terrorism, and the spread of fatal diseases, many advantages have been recorded. These methods have also been used to pinpoint disasters' epicentres and quickly dispatch aid.[3] provides a helpful discussion on social networking and emergency preparedness. Next, cyber attacks can target social media platforms. For instance, malicious software could modify the posted messages' content. Using such software, bogus profiles might be made and then used to post false information. Social media posts of photographs and videos could potentially come under attack. Finally, a significant portion of the social media system could become infected if the computers and mobile devices that social media users utilise are attacked. How can machine learning algorithms then identify such harmful behaviour is the question. There have been many reported attempts to use machine learning to find malware (e.g., [4]).Therefore, we must look into how to adapt such methods to the emerging attacks that target social media platforms. How to deal with fake news is a question that is connected. For instance, malicious software may lead to bogus news. However, the majority of the time, false stories about famous people being paedophiles or another famous person embezzling millions of dollars are spread by malicious human beings. It is quite difficult to identify such phoney news. Work has been done in this area [5].Training the machine models with multiple articles about an event or a person is what needs to be done. These pieces will unequivocally demonstrate that the subject is not a paedophile and is, in fact, a respectable person. The new articles must be tested after the model has been trained before it can decide whether or not the person is a paedophile based on the training. The news reports are always changing and coming in, which is the problem. As a result, several of the methods created for assessing developing data streams could be applied to the detection of fake news .Finding the source of the false information is another solution to the issue. Therefore, additional research into some of the data provenance solutions should be done for applications of fake news.

Social Media Security and Privacy

The social media networks could be exploited by malicious software, as was mentioned in Section II. For instance, a bad person could change what users post. The users' infected computers or the compromised content they post, including photographs and videos, could also be the source of the dangerous software. To identify such dangerous software, machine learning techniques are being investigated. Access control mechanisms have also been created for social media platforms [6].Fine-grained access to the social media data is made possible by these access control techniques. To further secure the users' identities, the proper methods of identification and authentication are required. Finding bogus users on social media platforms is one of their difficulties. These fictitious users do own valid email addresses. But they spread unpleasant rumours that are frequently made up and give misleading information about them. The issue is how to identify and stop fabled rumours [7].To identify such bogus profiles, machine learning techniques are being investigated. However, a significant issue is that the bogus accounts may be

made by malicious software and bots and may just slightly alter the information, but this could have a significant effect. Finding such malicious software presents a problem for cyber security researchers. The protection of people's privacy is another issue with social media platforms. Now, one could argue that the choice of what information to post about oneself is entirely up to the individual. However, occasionally people can over share and publish unrelated pieces of information about themselves, which taken as a whole may breach their right to privacy. The person might share pictures of holidays taken, say, in the Bahamas, which could prompt burglars to target the person's house. So, should the social media platform be in charge of asking the user if they actually want to disclose this information before going on to explain the potential privacy violations. Social media privacy issues are being researched [8], but we also need to know what is meant by privacy violations in these platforms. Exists a privacy safeguard. The inference problem is a closely similar issue where information may be highly classified in collections but unclassified in individual bits.By using inference controllers, we have created solutions to this issue [9].Should a social media system have an inference engine that can make assumptions about the material uploaded and then warn users when they publish new information that could breach security.

AI In Social Media Security

They talked about how bogus news and sentiment can be detected using machine learning approaches. Additionally, it covered how they might be used to find malicious software. Social media security and privacy issues were covered, along with some potential remedies. What are the security and privacy issues with applying machine learning techniques to social media systems, then The machine learning methods could be challenged first. In other words, the attacker might understand the learning model and attempt to undermine it. Then, the defender would modify the model. The adversary would become aware of the new design and attempt to undermine it. The attacker and defender then play a game against each other in this situation. Contrarian machine learning has come to be recognised as this [10].There has been a lot of recent research on this subject. However, we must look into how the suggested fixes may affect social media platforms. That is, how can we modify the machine learning methods being used to protect social media networks against cyber attacks. Machine learning-related privacy issues have been researched for almost 20 years .Machine learning can now be used to extract potentially extremely private information (or sensitive). Machine learning methods that protect privacy are being developed in many different ways. The difficult part is how to modify these methods for social media platforms. The problems and difficulties need to be further investigated notwithstanding considerable discussion in .Recently, "AI for Good" efforts have been launched by groups like the United Nations. Therefore, the problem we face is how to make AI work for good in the face of cyber attacks and privacy abuses .Moreover, how has social media affected AI for Good.

Social media

The advantages of social media and the use of machine learning techniques for social media have been covered in this essay. For instance, machine learning algorithms are used to gauge user sentiment, disseminate data on the development of lethal diseases, and stop child trafficking. It also covered the application of machine learning to the detection of harmful software and fake news. The article then examined access control models and privacy-aware social media systems as security and privacy concerns for social media systems. The integration of AI and cyber security for social media systems, including adversarial machine learning, inference, and privacy issues, was also covered in the article. Security and AI are just beginning to play a role in social media networks. We may anticipate further research on these AI applications in social media with the introduction of the AI for Good programme, Fair AI, and Bias in AI. The issue of using AI is complicated by cyber security breaches and privacy concerns. With cyber attacks and privacy abuses, for instance, how can AI be used for good on social media. How can adversarial machine learning be expanded for social media. How can we gauge social media privacy. How erroneous rumours may be recognised and stopped. There is still more work to be done even though some progress has been made.

Performance of AI Application Security Technologies

Machine learning, often known as artificial intelligence, has been heralded as the wave of the future for more than a decade. The term "artificial intelligence," sometimes known as AI, has undergone extensive development, and its creators have been working on it nonstop. To make things better and more dependable, AI is being included into

both already existent objects and those that are about to be created. As implied by its name, artificial intelligence (AI) is intelligence that has been demonstrated by computers that function similarly to humans and strive to accomplish the objectives that are given to them. AI may also be used to develop defences against current cyber threats, vehicle overrides, etc. Additionally, while though AI purports to be intelligent, it is ultimately only a collection of programmes, making it vulnerable to corruption or abuse on the part of others. It is essential to install the technologies with a resilient defensive system in order to prevent their misuse. There will undoubtedly be a default defensive system, but it is vulnerable to hacker corruption or intelligence breakdown in specific situations, which might have fatal results, especially in the case of robotics. The following paper offers a solution for safeguarding artificial intelligence by introducing a concept known as "Guard Masking."

Artificial Intelligence's Applications

Threats to artificial intelligence's integrity arise from its own growth since hackers frequently want to access devices that make human existence better. Maintaining and protecting its integrity is crucial to preventing any threats. It has numerous applications, and more are being added daily to help us live better lives. These days, AI is also in charge of providing security, safeguarding our data, identities, and work. A minor alteration to the algorithms could result in severe AI corruption, which could have drastic consequences. Therefore, in order to keep it stable and retain its integrity, it is necessary to work on its own defensive strategies as well. To secure AI and our data, new methods and systems must be developed. Guard Masking is currently merely a notion, but it might be a reliable temporary solution. The idea needs to be developed further before being put into practise, thus it can't be done right immediately. Guard masking will be correctly implemented as part of our future plans. It is currently only an idea and needs several changes before being put into practise. In the future, we won't simply focus on improving Guard Masking; we'll also take the time to learn more about the weaknesses in security protocols in order to create something more dependable and secure. We are always open to ideas and assistance in putting the notion into practise.

The Function of AI and Cyber security in Social Networks and the Internet of Things

Knowledge has been disseminated through social media platforms, which has the potential to be very harmful to people and their reputations. Cyberattacks may also compromise the social media platforms and the methods used for analysis, which may compromise the posted information. The Internet of Transportation network is another form of network where AI and cyber security are being used. With the development of the internet, it is now possible to gather, store, manage, and analyse huge volumes of sensor data coming from many different devices and sensors, including those used in different transportation systems. The Internet of Transportation Systems refers to all such systems taken together. The Internet of Transportation Planning refers to these systems as a whole. The Internet of Transportation and the infrastructures that enable it face a dilemma with regard to security and privacy. Traditional cyber security methods like encryption are ineffective for securing the Internet of Transportation because of the massive amounts of heterogeneous data being gathered from multiple devices. There are some promising physics-based solutions currently being developed. There will be two sections to this keynote address. The first section investigates the use of artificial intelligence (AI) tools for social media and looks at how social media platforms could be safeguarded against online attacks. Additionally, the potential invasions of privacy are also discussed. The talk will also go through some of the new issues that have arisen as a result of the spread of fake news and cutting-edge cyber attacks on social media platforms. The presentation's second section looks at the progress being made to secure the Internet of Vehicles and the infrastructures that enable it, as well as the ramifications for privacy. Finally, it discusses how the Internet of Mobility and Infrastructures could include AI and Security.

An analysis of the numerous problems with artificial intelligence in cyber security

Artificial intelligence's use in cyber security has a double-edged effect; while it can significantly increase security, it also raises the possibility of new types of attacks that can be launched against AI itself. Machine learning algorithms have shown to be effective at spotting zero-day attacks and identifying unexpected system behaviour that could be a sign of malware or an assault. This study examined a variety of security risks, countermeasures, and open issues in the field of cyber security for intrusion detection, malware detection, and network anomaly detection systems

utilising a variety of machine learning and deep learning methods. The majority of the approaches presented were found to involve supervised models. RBF-SVM (Radial Basis Function - Support Vector Machine) model provided the best accuracy for intrusion detection (99.90%), while DNN (Deep Neural Network) model provided the highest accuracy for malware detection (97.79%).A DNN model was employed to identify pirated software, and it provided accuracy of 96%.The Seq2Seq (Sequence to-Sequence) model, which had a 99.90% accuracy rate, functioned best for detecting network anomalies.On the other side, a DBN (Deep Belief Networks) based model is employed for anomaly detection and provides 69.77 percent accuracy. Finally, this article covers the security of 5G, cyber attacks, and the significant significance of the aforementioned growing industries in the future of cyber security.

CONCLUSION

Attacks are becoming more and more complex today, and new attack types are being created daily. When it comes to known attacks, our present security mechanisms perform admirably, but occasionally new kinds of attacks render them ineffective. If given enough data, artificial intelligence systems can do well at anticipating such attacks so that we can thwart them. This has been lot simpler, especially with the emergence of Deep Learning, as they are adept at handling massive amounts of data. The majority of IT and network systems generate a lot of data every second, making it simpler for us to collect enough data to build efficient neural networks. This paper covers previous work on the topic and addresses the use of AI-based systems in detecting such attacks. Additionally, it discusses how cyber security can protect AI from attacks aimed at its judgement and model correctness. In addition, it demonstrates what additional research might be done in the future to strengthen and improve such systems.

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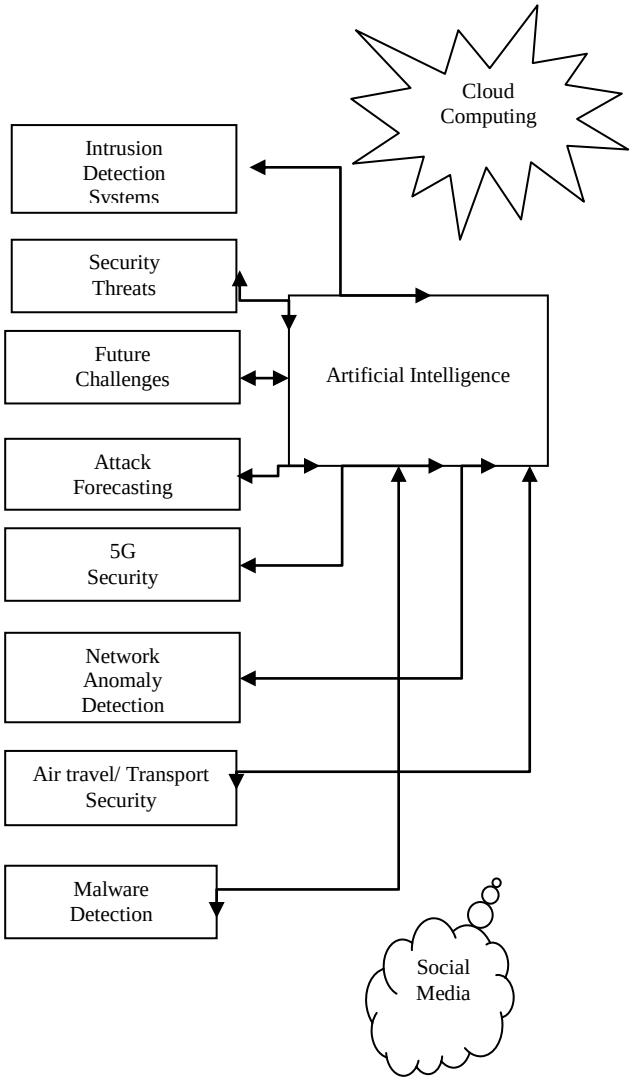

Fig 1 Block Diagram Artificial Intelligence in Social Media

REVIEW ARTICLE

Ethnobotanical Survey among Irular Tribes in Krishnagiri District, Tamil Nadu, India – A Review

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ABSTRACT

Ethnobotany is a distinctive branch of natural science that deals with various aspects such as anthropology, archaeology, botany, ecology, economics, medicine, religious, cultural, and several other disciplines. Ethnobotany is generally defined as an anthropological approach to botany. There are many methods of ethnobotanical research and those relevant to medicinal plants are archaeological searches in literature, herbaria, and field studies. Recently ethnobotanical studies have gained importance during recent years. In the present paper ethnobotanical surveys have been conducted among Irular tribes. This paper examines and evaluates the ethnobotanical data currently available on medicinal plants traditionally used for human and livestock of Irular tribes of Krishnagiri District, Tamil Nadu. 220 ethnomedicinally important plant species are found in the survey, among which 110 (50%) tree species, while there were 49 (22.3%) herb species, 29 (13.2%) shrub, and climberspecies and 3 (1.4%) lianas species. These plants are used to treat general health problems and some plants are used to treat major health problems and sexual problems in women and men. Some plants are also used in veterinary. Large proportions of the medicinal plants were threatened by anthropological activities. Most often, knowledge is passed down from ancestor to their generations.

Keywords: Traditional medicines, Health problems, Herbs, Irular tribes.

INTRODUCTION

Evolution of human life and culture has direct or indirect association with nature, and influenced by the surrounding environment with the results of changes in life style. Ancient men lived closely associated with nature chiefly depended on forests for survival. Their dependence on the plants around them made them acquire the knowledge of economic values of many plants and animals by trial and error and consequently they became the storehouse of knowledge of many useful as well as harmful plants, accumulated and enriched through generations. Von Furer-Haimendorf, Christoph (1992), in his book '*Tribes of India: The struggle for survival*' has mentioned that, one phenomenon inherent in the nature of the plural society of the Indian subcontinent is the coexistence-often in narrow space-of populations varying greatly in the level of material and intellectual development. The biodiversity of India has varied type of habitats and climate along with rich traditional knowledge among different tribal communities. India's traditional medicine systems have the potentials to capture the world drug and pharmaceutical markets by making value addition to the existing traditional knowledge base through appropriate scientific and technological intervention and policy support (Pushpangadan, 2002). Several tribal communities are surviving in different habitats of India, the survival of their traditional knowledge is questionable. The traditional knowledge related to the use of natural resources is inherited through generations by the tribal communities (Johari and Karki, 1999).The tribes are rapidly being assimilated into modern societies and their traditional knowledge is fast disappearing. Before this rich unwritten folklore on uses of plant resources is lost forever, it must be properly documented and preserved. Although several ethno-botanists and anthropologists have made attempts to document the traditional knowledge in various parts of the world, several remote localities and indigenous communities have remained unnoticed (Raut*et al.*,2012).

Ethnomedicine and Drug Discovery

Ethnobotanical studies are proving to be powerful tools in the search for new drugs. However, it is necessary to recognize that the relationship among people, their traditions, and the use of natural resources for medical purposes can be quite complex. Albuquerque (2010) has shown the implications of some findings that may be relevant to the search for new drugs in semi-arid regions. Eighty percent of the world's population depends on plants as their main source of medicine (Plotkin2000). Ninety plant species provide 120 therapeutic agents for commercial pharmaceuticals (Farnsworth and Soejarto1985). The National Cancer Institute has identified 3,000 plants active against cancer cells (Cragg*et al.*1997). One third of the U.S. population spends at least $3.5 billion on herbal medicines each year (Tyler,1996).

Ethnobotany in India

The elements of Ethnobotany in India can be unearthed even before the definition of this science by Harshberger (1895). Patil (2012) reviewed the science to initiate debate among the Indian ethnobotanists and to chalk out justifiable strategy for India. Less well known ethnomedicines have been identified that are used to treat intestinal, joint, liver and skin diseases (Jain, 1994).'Chatara' block of district Sonebhadra in Uttar Pradesh State, India showed the presence of 156 ethnomedicinally important plant species belonging to 63 families. Some of the important plants are declining because of overexploitation and environmental disturbances (Singh *et al*., 2010). Namsa *et al.* (2011) documented 50 plant species belonging to 29 families used for treating 22 human and 4 veterinary ailments. Padal *et al.* (2012) provided the ethnomedicinal data for 95 taxa of plants belonging to 45 families, particularly to cure endemic diseases of local tribes of Munchingiputtu mandal of Visakhapatnam District in Andhrapradesh, India. A critical analysis on the trading system, by Yumkham and Singh (2013) showed that womenfolk dominated the entire workflow of activities like harvesting, transportation of plant materials from forests, and even regulating seasonal market prices. Mao *et al.* (2009) have reviewed the plant wealth of Northeast India (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tirupura) with reference to ethnobotany which includes about twenty-five species of threatened plants. There are 200 plant species from Arunachal Pradesh for the treatment of 44 different diseases and ailments, 286 plant species from Assam for the treatment of 40 different disease and ailments 526 plant species from Nagaland for the treatment of 83 different diseases and ailments and 194 plant

species from Tripura for the treatment of 50 diseases and ailments (Hynniewta, 1984, 1987). 834 medicinal plants have been recorded from Meghalaya by Foundation for Revitalization of Local Health Tradition (FRLHT), Bangalore (2006).The medicinal plants used by *Paliyars* are listed with Latin name, family, local name, parts used, mode of preparation and medicinal uses. Generally, fresh part of the plant was used for the preparation of medicine (Ignacimuthu*et al.*,2006).A floristic survey of ethnomedicinal plants occurring in the tribal areas of Kaladera region in Rajasthan has been conducted by Pareek and Trivedi (2011) to assess the potentiality of plant resources for modern treatments based on the exhaustive interviews with local physicians practicing indigenous system of medicine, village headmen, priests and tribal folks. Sathyaraj*et al.* (2012) have reported 25 species of plants used as antifertility agents by the local people in Kathiyavadi village, Vellore District, Tamil Nadu, India. Baruah and Borthakur (2012) have studied on morphology and ethnobotany of six species of *Garcinia* L. (Clusiaceae) found in the Brahmaputra Valley, Assam, India. Members of the genus *Garcinia* L. is known for their edible fruits, and medicinal properties. *Garcinia* L., commonly known as "*Thekera*" by Assamese people.Results of an ethnobotanical study of wound healing treatments among the tribal people of Tirunelveli hills in southern India are presented by Ayyanar and Ignacimuthu (2009) which comprises of 46 plants belonging to 44 genera and 26 families have been documented for their therapeutic use against wounds and related injuries such as cuts, burns, bruises caused by external injury, boils, sores, abscess and wounds created during delivery.According to Das*et al.* (2009), 33 ethnomedicinal plants belonging to 32 genera under 25 families are used by ethnic people of Tripuri and Reang communities of Tripura State, India. Ethnobotanical information has been provided for coastal Andhra, Rayalseema and Telengana. Neelima*et al.* (2011) have made ethnobotanical studies in Rapur forest division of Nellore district in Andhra Pradesh. Traditional uses of 124 plant species belonging to 40 families are described for their medicinal properties from the Vellore district of Tamil Nadu by Sundaresan and Senthilkumar (2013). Ethnobotanical uses of plants in Himachal Pradesh, Northwest Himalayas (Sharma and Devi, 2013) shows the presence of 55 medicinally important species with 20% for stomach disorders, 17 % for skin complaints, 14 % for asthma, 11 % for fever and joint pains, 3 % for aphrodisiac and snake bite and 1 % for anti-cancerous and nerve disorders.

Ethnobotany of Eastern Ghats, India

The Eastern Ghats constitute an important bio-geographic region, as one of the nine floristic zones in India and form a broken chain of mountains spreading along the states of Orissa, Karnataka, Andhra Pradesh and Tamil Nadu and lie between Mahanadhi and Vaigai rivers. Muthumperumal and Parthasarathy (2009) provided a check list of angiosperms climbing plant species, along with their climbing modes, enumerated from a total of one hundred and fifty grids in tropical forests of southern Eastern Ghats, peninsular India. Medico-ethnobotanical study on Yercaud Hills by Parthipan*et al*. (2011) resulted about 48- plant species belonging to 45 genera and 29 families of medicinal plants related to folk medicine used by the local people. Elavarasi and Saravanan (2012) have recorded sixteen species of plants under fourteen families to treat diabetes by the tribal people in Kolli Hills, Eastern Ghats, Tamil Nadu. 89.3 percent women in Krishnagiri District, Tamil Nadu, used *Vetiveria zizanioides* (Vetiver) for boils, snakebite, fever, epilepsy, rheumatism and sprain. 32 percent opined that *Phyllanthus amarus* (Kellanelli) for antidote for the snakebite. 78.6 percent mentioned, *Madhuca longifolia* (Illupai) oil will promote the longevity of people. 63.7 percent mentioned *Justiciaadatoda* will cure bleeding piles and bronchitis. To reduce excess thirst while hunting, 42 percent opined that, *Piper cubeba* (Valmilagu) can be chewed. For colicky pain and intestine cramping 74% explained that *Terminalia chebula* (Kadukai) may reduce the pain. 64.5 percent mentioned that powdered form of *Azadirachta* (Neem leaves) and *Liliocerislilli* (beetal leaves) will prevent the thread worms (Malathi and Kala, 2012). Nutritional and antinutritional studies on two samples of seed materials of the underutilized tribal pulse, *Dolichos lablab* var. *vulgaris* (dark brown and pale brown coloured seed coat) collected from Anakodi, Krishnagiri district, Eastern Ghats, Tamil Nadu showed that the tribal pulse is a good source of protein, essential amino acids, essential fatty acids, minerals and vitamins. All the anti-nutritional factors reported except L-DOPA are heat liable (Kalpanadevi and Mohan, 2013). Niyamgiri Hills (Eastern Ghats-Orissa), the abode of the primitive DongriaKandha tribe in southwest Orissa, is a unique forest ecosystem harbouring a rich flora and vast natural resources. 20 species of orchids are used by the Dongarias of the Niyamgiri hill range to treat 33 kinds of diseases (Dash*et al.*2008). Britto and Mahesh, 2007, has done the ethno medicinal survey of medicinal utilization among Kani hakims 76 species of plants distributed in 64genera belonging to 43 families have been reported. Similar to the present study works have been done earlier by

Kalaiselvan and Gopalan 2014; Khan *et al.,* 2014; Upasana and Bharti, 2015; Meragiaw*et al.,* 2016; Zambrana*etal.,* 2017 about the tribals. As stated by Idu (2009) the ethnobotanical information may be of more valuable when the use of same plant is recorded from different locations by the same or different communities. Eastern Ghats in India is an important phytogeographic region with rich biodiversity and varied tribal communities. But there are only sporadic reports on ethnobotany of different tribes from this phytogeographic region (Dash*et al.*2008, Parthipan*et al*., 2011, Elavarasi and Saravanan, 2012, Malathi and Kala, 2012; Yumkham and Singh, 2013; Kaval *et al.,* 2014; Mukemre*et al.,* 2015; Ballesteros *et al.,* 2016; Fongnzossie *et al.,* 2017). So, the present study has been mainly aimed to make ethnobotanical survey among Irular tribes from the Hosur Forest Division of Krishnagiri District, Tamil Nadu, India.

Ethnobotany of '*Irula*' Tribes in India

Irulars in Tamil Nadu constitutes 1% of total India's tribal population. They are dravidian tribe found in the states of Tamil Nadu, Kerala, Andhra (Chittoor) and Karnataka. The word 'Irular' derived from Tamil word called "Irul" which means 'darkness'. 'Irular' means those who are in darkness. Curly hair and dark complexion is their prominent feature (Sinu, 2013). One of the Largest Tribes in Tamil Nadu is Irulas and it has been facing several psycho-social-economic problems during last two decades. Earlier they were traditionally snake trappers, with the ban on trading snake and its skins without any alternative rehabilitative measures their living conditions of their life has been affected. Recent study by Sinu (2013) on the living conditions of Irula tribes of Gingee taluk, Villupuram district, Tamil Nadu has shown66% illiteracy, negative attitude towards education and girl children education, (84%) inadequate housing conditions, majority involved in farming, job insecurity, low income, indebtedness, 81% under below poverty line, without using banking facilities , absence of toilet facility at home, most of them do not have community certificate to avail government welfare measures, alcohol dependence is seen among men folk, poor quality of life, poor health care facility and overall poor living condition. The study also revealed that they live in joint family, do not promote dowry, inter-caste marriage, men and women enjoy equal social status, live in harmony and actively participate in self-help group, temple festivals along with other community. Hosagoudar and Henry (1996) have studied the ethnobotany of tribes Irular, Kurumban and Paniyan of Nilgiris in Tamil Nadu, Southern India with several new reports. Ragupathy and Newmaster (2009) tested consensus (reliability / replicable) of Traditional Knowledge within the Irulas of the Kodiakkarai Reserve Forest (KRF), India. They have calculated relative frequency (RF) and consensus factor (F_{ic}) of TK from 120 Irulas informants knowledgeable of medicinal plants. Their research indicates a high consensus of the Irulas TK concerning medicinal plants. The Irulas revealed a diversity of plants that have medicinal and nutritional utility in their culture and specific ethnotaxa used to treat a variety of illnesses and promote general good health in their communities. Ethnobotanical survey carried out by Revathi and Parimelazhagan (2010) among the Irula ethnic group in Hasanur Hills (Southern Western Ghats) revealed that the tribal's use 70 wild valuable plant species belonging to 42 families.

The common diseases treated by the herbal practitioner were asthma, digestive problems, paralyzes, skin diseases and diabetes. Extensive field surveys conducted in the two Irulars villages namely Erumavettipalayam and Thirunilaicolony involving 10 households in the Red Hills of Chennai District, Tamil Nadu shows the ethnobotanical uses of 35 plant species under 27 families (Bosco and Arumugam, 2012). In the Papillionaceous family, 5species are adequately used in the preparation of ethno medicine followed by Acacnthaceae, Poaceae, Malvaceae, Euphorbiaceae and Zingiberaceae (each with two species) and the rest of the families have only one species each. Jeerango Gram Panchayat, the district head quarter of Gajapati, under Mahendragiri hill system of Eastern Ghats with an area of 5996 hectares comprising of 16 villages with a total population of 4194 has been surveyed ethnobotanically by Senapati*et al* (2010). They have given ethnobotanical information for sixteen species of plants along with ethnozoological information for two mammals. An ethnobotanical survey carried out among the ethnic groups (Kani / Kanikaran) in Southern Western Ghats of India shows the traditional uses of 54 plant species belonging to 26 families and several plants are mostly used to cure skin diseases, poison bites, wounds and rheumatism (Ayyanar and Ignacimuthu, 2005a). Sekhar *et al*. (2011) have made an ehtnobotanical survey among various tribes including Yanadis or Irulas of Chittoor district in Andhra Pradesh. They have recorded 62 species belonging to 37 families. Most of the ailments are noted from Fabaceae family. Ethnobotanical studies carried out in ethnically different groups of Eastern Ghats of Andhra Pradesh and Orissa during 1997 to 2006 by Reddy*et al.* (2006)

has resulted in the collection of information on 28 endemic plant species used by local ethnic groups namely: Bagatas, Chenchus, Gonds, Kondareddis, Koyas, Lambadas, Nukadoras, Valmikis, Yanadis, Yerukalas of Andhra Pradesh and Kondhas, Gadabas, Sauras, Didayis and Kolhas of Orissa. Umapriya*et al*. (2011) have reported fifty ethnomedicinally important plants under 47 genera used by Irular tribe in Palamalai Hills, Coimbatore, Tamil Nadu. These plants are commonly used to treat several common diseases such as skin diseases, dysentery, cough and cold, cuts and wounds etc. The use of *Coccinia indica* and *Pongamia pinnata* for diabetes, *Vitex negundo* for cardiac disorders, *Carica papaya* and *Indigo feratinctoria* for poisonous snake bites, *Cardiospermum halicacabum* for rheumatic pains, *Cassia auriculata* for scabies and bone fractures are some of the interesting findings of them. Kanthasamy *et al*. (2016)carried out a survey of plants of irular tribes in krishnagiri district showed that 58 species of plants distributed in 55 genera belonging to 28 families were found on this reason for treating various ailments. In the modern world, urbanization and movement of people to urban area are common. Tribal people also move from the wild forest to some other areas either on their own interest or due to the compulsion by the Government. Such kind of mixture among culturally different population may lead to change in their life style, including ethnobotanical uses of plants. Anthropologists may be interested in such change among population. Interestingly, a total of 87 plant species have been reported by Ososki *et al.* (2002) in the Dominican literature for the conditions and symptoms among women, such as uterine fibroids (benign tumors of uterine smooth muscle); menorrhagia (excessive uterine bleeding); endometriosis (growth of endometrial tissue outside of the uterus); and hot flashes (sudden brief sensations of heat commonly experienced during menopause). Nineteen species overlapped from the literature survey and the fieldwork with Dominican healers in New York City, representing 29% (n=65) of the plants prescribed by healers in New York City. This study offers a model to investigate changes in plant use as people migrate to urban centers where they are surrounded by diverse cultures, healing systems, and new environments.

Ethnobotanical Survey of Medicinal Plants used by Malayali Tribes in Yercaud Hills of Eastern Ghats, India showed the uses of 90 species of plants distributed in 80 genera belonging to 44 families (Senthilkumar *et al.* 2013). Ethnobotanical survey made on the utilization of medicinal plants among the people of selected six villages from Jawadhu hills of Eastern Ghats in Tamil Nadu, carried out by Ranganathan et al. (2012). This work revealed the use of forest plants, weeds, fruit plants, vegetables, spices, ornamental plants, ferns and many others as traditional medicine. Although many of these species are known as medicinal plants, others are mainly used for non-medicinal purposes such as preparing agricultural implements. Santalum album, Terminalia bellirica, Cassia fistula, Gymnemasylvestre, Melia dubia and Rauvolfiatetraphylla are the leading species used as remedies against a variety of complaints. Rao and Pullaiah (2007) have reported ethnobotanical uses of twenty-nine rare and endemic plants from Eastern Ghats India. Recently, Thirunarayanan (2013) has reported forty-nine plants with the folk medicinal importance in the management of animal bite poisons in the forest tract of Salem region of Tamil Nadu, India. The most commonly used species is *Aristolochia bracteata*. Most of the plants used by the healers also find reference in the classical texts of the codified systems of medicine. Ethnobotanical studies about the wild edible plants collected and utilized by the Irula tribes of Pillur valley, Coimbatore District, Tamil Nadu, India shows the usage of 74 plant species with 42 fruits yielding plants, 26 green leaves, 7 tubers, 4 young shoots and 2 flowers (Rasingam, 2012).`The Irulars in Anaikatty hills, Coimbatore district, Tamil Nadu use about 89 species of plants as food and for medicinal purposes. The elder generation has sound knowledge about these plants. Due to modernization, the tribal community is changing their life style (Geetha *et al.* 2007). Like that *Begonia laciniata, Dendrobium ovatum* and *Cuscuta epithymum* are traditionally used as hepatoprotective medicinal plants by the herbalists across Kuppam, Sathupally and their surrounding villages of Chittoor and Khammam districts of Andhra Pradesh, India (Ganapathy *et al.*2013). From the above review it is clear that India is not only rich in bioresources, but also rich in traditional knowledge with different tribal communities, particularly Irular tribes throughout India.

DISCUSSION

The main objective of the present study is to make intensive ethnobotanical survey among the Irula tribes in Krishnagiri district, Tamil Nadu in order to know their knowledge resource about the local plants. By making

frequent field visits ethnobotanical information was gathered personally by meeting the Irula tribes. Irula tribal community is one of the major hill tribes inhabiting in Krishnagiri District. There are about 125 settlements, 2400 families with population of about 10,454. In Krishnagiri District of Tamil Nadu, Lingaith and Irulas are the traditional hill dwellers who follow mixed culture from Tamil and Kannada. Lingaiths worship male god Easwaran (version of Siva) as their traditional God while Irulas worship female god Mariyamma (version of Parvathi) as their traditional God. Marriage among the Irula is purely by mutual consent. The ceremony is not indispensable although it is becoming fashionable nowadays. A man may take several women at a time, but a woman may take only one at a time. Divorce is easy and frequent. When a girl attains puberty, she is placed in a separate hut erected then and there for eight days. A stick of the *Strichnosnux-vomica* plant is placed inside the hut to ward off evil spirits. On the ninth day, the girl bathes and free from evil sprits. The hut is burnt down. Through ethnobotanical study among Irula tribes in Hosur Forest Division of Krishnagiri District, Tamil Nadu, India, 220 species of ethnomedicinal plants distributed in 182 genera belonging to 74 families have been recorded along with methods of preparation and administration. Majority of the plants are used to treat general health problems and considerably several plants are used to treat major health problems and sexual problems in women and men. To treat skin-health problems including dandruff, several plants are in record. Some plants are also used in veterinary. Comparatively few plants are edible. For miscellaneous uses there are several plants. Among 220 ethnomedicinally important plant species is found in survey, among which 110 (50%) tree species, while there were 49 (22.3%) herb species, 29 (13.2%) shrub and climbers' species and 3 (1.4%) lianas species. It is remarkable to note that more than fifty percent of the graphed species are trees.

CONCLUSION

Irular tribes of the Hosur Forest Division in Krishnagiri District, have enormous knowledge to treat all kinds of health problems both common and major diseases and disorders including sexual disorders / problems. Irula tribes used the bark of the tree *Erythroxylum monogynum* roxb .The bark is burnt and mixed with sandal wood oil and the mixture is applied on skin for 7 days in the morning and evening followed by bath in hot water to cure skin disease. This is the first report for this species. The uses of several ethnomedicinal plants of the present study have already been proved pharmacologically and some species are yet to be confirmed pharmacologically. Among 220 ethnomedicinally important plants, only two species (*Cycas circinalis* L. and *Shorea roxburghii* G. Don) are under endangered list of IUCN. Recent report on reproductive biology of *S. roxburghii* shows that non-annual flowering, massive flowering for a short period, high bud / flower and fruit infestation rate, absence of seed dormancy and rocky habitat could attribute to the endangered status of *S. roxburghii.* An efficient protocol for mass-clonal propagation of *Shorea roxburghii* by shoot-apex culture has been developed by Nakamura (2006). In contrast, the endangered nature of the endemic species *Cycas circinalis* L. in India is mainly due to over harvesting of different parts of the plant by the local people. So, conservation measures driven by local communities are vital to create management plans for the sustainable use of this endemic species.

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REVIEW ARTICLE

Machine Learning Perspectives in Agriculture Ecosystem: A Review

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ABSTRACT

Data-intensive processes in modern smart farming may now be quantified and better understood because to the emergence of the rising notion of machine learning, big data, and high-performance computing. When it comes to farming, machine learning is now a major component in every step of the process, from the initial soil preparation to the seed breeding and water feed monitoring, to the robots that pick up the crop and determine its readiness using computer vision. Crop management, soil management, animal management and disease identification may all benefit from machine learning. This paper provides a thorough review over different applications of machine learning in agriculture ecosystem.

Keywords: ANN, CNN, Agriculture ecosystem, machine learning, deep learning.

INTRODUCTION

Farming, animal husbandry, and fishing industries produce artificial ecosystems by constructing farmland and coastal/aquatic zones, also known as agriculture ecosystem. Human cultures have benefited greatly from these ecosystems since agriculture was first developed, and this trend will continue. People have relied on it for years as their principal source of food, income, and other fundamental necessities. In the Philippines, it has traditionally been one of the country's most important economic engines [1]. Over a third of the 1,210 species of locally cultivated agricultural plants have food value[2]. Other cash crops for feed, medicinal/herbal, decorative, and industrial purposes are also supported by agriculture. The Philippines' primary agricultural products include rice, maize, and coconut [3]. Agricultural ecosystems, despite their undeniable socioeconomic significance, are both endangered and

act as a hazard to other ecosystems. Continuous urbanisation for residential and commercial purposes is encroaching on agricultural fields[4]. Forest ecosystems are damaged as a result of farmers being compelled to relocate. The so-called Green Revolution of the 1960s spawned a controversy about high-value "hybrid crops and genetically modified organisms" that has to be addressed in agriculture. There is a lot of debate about whether or not modern biotechnology applications are being developed with the benefit of humanity in mind ("i.e., increasing food supply and ensuring global food security"), but the environmental and human health risks associated with the propagation of these crops have not been adequately studied [5].

Characterizing the Agriculture Ecosystem

Abiotic (non-living) and biotic (living) components make up agricultural ecosystems in a human-managed system. Crop evolution takes place in agricultural environments, which offer crops and farmers with both stress and opportunity[6]. Environments in the agricultural sector are characterised by abiotic factors such as temperature and soil moisture. There are biotic variables such as "parasitic and herbivorous pests, competition between crops and other plants", and beneficial (symbiotic) connections among species, such as belowground creatures and pollinators[7]. Farming methods like as irrigation, fertiliser inputs, pest management, "land preparation, mixed/relay cropping", and others are also "biotic components" of agriculture ecosystems [8].

There are four key differences between this ecosystem and one found in nature[9]:

- Simplification: All other animals and plants are exterminated from an area in order to protect a single kind of plant
- A man's energy intake is represented by technology, fertilisers, insecticides, carefully chosen crops, and other methods of production.
- When the harvest is mature, the biomass is removed. When external activities reintroduce, fertilising chemicals suited for nourishing a fresh "growth and development process" of organic material, the ecosystem becomes an open system (plants). A natural ecosystem, on the other hand, reproduces itself since the biomass is left in the same location where it was found originally.[10]
- When chemical fertilisers, anti-parasitic ides, and other non-biodegradable chemicals are introduced into the environment or seep into the subsoil, they pollute groundwater, seawater, and rivers. This is especially true in intensive agriculture [11].

A house may be thought of as a micro-ecosystem all by itself. Solid and liquid waste created by human activities is taken from the outside of homes and brought inside, where they may be used for food, energy, and water. Similarly, the city operates. Waste created in cities is put outside the urban area in landfills and incinerators; this implies that anything that does not contribute to the survival of the urban ecology has been removed from the city.

Components of Agriculture ecosystem

Primary producers: Agro ecosystems are primarily supported by the food that crops and weeds in the field provide. As an example, in a rice field, there are a number of rice producers such as "durba, mutha, and syma" as well[12].

Consumers

Macro-consumers include grasshoppers, aphids and beetles; micro-consumers include frogs and snakes.

Properties of Agriculture ecosystem

Productivity: Value added per unit resources ("land, labour, energy and capital") is referred to as yearly yield /hectare and is often expressed as a percentage.

Stability

It is referred as the degree till which productivity remains stable, despite the usual fluctuations in external factors such as temperature or the economic state in the market.

Sustainability

Stress tolerance refers to a system's capacity for sustained production in the face of adversity. Unpredictable and generally small-scale perturbations that occur on a regular basis are what we mean by "stress." Increasing soil salinity, for example. Disruptions such as drought or flood are known as perturbations, while new pests or disease outbreaks are known as perturbations.

Equitability

It is referred as the measure of how equally the Agro ecosystem's human beneficiaries benefit from its product. Equitable systems distribute food more fairly among the people of a farm, a hamlet, a region, or a country.

Machine learning in agriculture ecosystem

New prospects for unravelling, quantifying, and understanding data heavy processes in agricultural operational contexts have evolved as a result of machine learning, big data technology, and high-performance computing. Machine Learning is the scientific subject that enables computers to learn without being explicitly programmed. Big data and high-performance computers have combined to generate new potential for unravelling, quantifying, and understanding data heavy processes in agricultural operating contexts. At each step of the agricultural process, we'll examine how Machine Learning may help:

Species management

Species Breeding

This application is one of our favourites since it is both rational and surprising at the same time. Usually, prediction of the harvest managing the ambient conditions are discussed later in the process. It is a lengthy process of looking for genes that influence water and nutrients usage, adaptability towards resisting the disease, varying climate and it nutritious contents or a superior flavour. Machine learning, particularly, “deep learning algorithms”, use decades of field data to study crops' performance in diverse climates and to discover new features that have arisen as a result of this analysis. To construct a model that predicts which genes are most likely to be helpful to a plant, scientists use this data.

Species Recognition

The vein morphology of a leaf provides more information about the leaf's qualities than colour and shape, and machine learning may deliver more accurate and quicker results by examining this vein morphology.

Field conditions management

Soil management

For agricultural experts, soil is a complicated natural resource with a myriad of processes and ambiguous mechanisms at play. Just by measuring its temperature, we can get a sense of how climate change is affecting the region's agricultural output. Understanding how ecosystems and agriculture are affected by evaporation processes, soil moisture, and temperature is the goal of machine learning algorithms.

Water Management

The hydrological, climatologically, and agronomic balances are affected by agricultural water management. It is possible to operate irrigation systems more efficiently and better anticipate the daily dew point temperature using machine learning-based apps that have been created so far. These applications may help detect predicted weather events and estimate evapo transpiration and evaporation.

Crop management

Yield Prediction

In precision agriculture, one of the most significant and often discussed issues is yield prediction, which deals with yield estimate, crop supply and demand matching, and crop management. Farmers and the general community

benefit from state-of-the-art techniques that use computer vision technology and "multidimensional analysis of crops, weather, and economic situations" in order to maximise yields.

Crop Quality

The value of a product may be increased and waste reduced by accurately identifying and classifying crop quality attributes. Machines, as opposed to human specialists, may make use of apparently useless data and relationships to expose and discover new features contributing to the overall quality of crops.

Disease Detection

Even in greenhouses, the most common method of pest and disease management is to spray insecticides evenly throughout the growing area. This method's effectiveness necessitates the use of large quantities of pesticides, which is both expensive and harmful to the environment. Management of agro-chemicals input is targeted in terms of time, location, and the impacted plants when using ML.

Weed Detection

Weeds are the second most significant danger to agricultural yield, behind illness. Detecting and distinguishing weeds from crops is the most challenging part of weed control. Weeds may be detected and differentiated using computer vision and machine learning techniques at a minimal cost and with no environmental or adverse effects. In the future, robots will be used to eliminate weeds, reducing the need for pesticides.

Livestock management

Livestock Production

Using machine learning, which is similar to crop management, it is possible to accurately forecast and estimate agricultural factors, such as cow and egg production, in order to maximise economic efficiency. For example, 150 days before the slaughter date, weight prediction systems may anticipate future weights, enabling farmers to change diets and circumstances.

Animal Welfare

Livestock are increasingly being viewed as more than simply a source of food in today's world; they are also seen as creatures that may be dissatisfied and worn out by their existence on a farm. As a result of their chewing and movement patterns, animal behaviour classifiers are able to relate the animal's requirement for a change in food to its level of stress and may forecast its susceptibility to illnesses, weight increase, and productivity.

Models of machine learning used for agriculture ecosystem

"Artificial and Deep Neural Networks (ANNs and DL) and Support Vector Machines (SVMs)" are the most used models in agriculture (SVMs). Pattern formation, cognition, learning, and decision-making are all simulated by ANNs, which are based on a reduced representation of the biological neural network's structure. In the detection of weeds, illnesses, and other pests and diseases, these models are often employed for regression and classification tasks. Since the recent evolution of ANNs into deep learning, ANN applications have grown to include agriculture. A linear separating hyper plane is used to generate a binary classifier called an SVM. Classification, regression, and grouping may all be accomplished using SVMs. Crop output and quality, as well as animal production, may be predicted using these models in farming. Multiple classifier systems in ensemble learning or Bayesian models—probabilistic graphical models where the analysis is done within the framework of the Bayesian inference—are necessary for more complex tasks like animal welfare evaluation.

LITERATURE REVIEWS

Drip Irrigation System

An upgraded NN as well as genetic algorithm back propagation was employed in the article to examine the irrigation capabilities of neural networks, as well as the influence of major irrigation water model technologies. It

uses an improved BP neural network algorithm that predicts maize yields for different drip irrigation methods. In this case, the forecast was accurate, and the standard deviation of the error was lowered.[13]. It depicted the link between irrigation and crop yields in vivid detail.

Maize Plant Detection

Weed control in maize farming using neural network back propagation is the topic of the research. Weed management is a time-consuming and expensive part of farming. Tests on photos with varied light conditions and without apparent geometric patterns show the suggested structure to be effective in these situations. The BPNN findings were encouraging since physical isolation of the maize plant from other dangerous weeds takes time.[14]

A lot of land is needed to grow enough maize to feed the world's population. Sunlight is essential for the health of crops, and it may be grown in a variety of ways. After a minimum of one month, the crop's changes may be seen and it is now safe to consume. SVM and ANN algorithms were presented to increase prediction accuracy and performance efficiency as a result of this research. Desktop learning is used to assess the suggested system's performance efficiency by comparing the training set values. Photo courtesy was used to detect plant disease in the SVM [15].

Viticulture

A look at the potential of NN in viticulture is presented in this study, along with an explanation of the impact that technology has had on the wine industry as a whole. Agriculture in the Mediterranean region has long used ANN approaches to enhance agricultural operations and better understand the interaction between the farmers and environmental elements, including as climate and soil, that affect the quality of their produce[16]**.**More than two decades of study have been dedicated to better understanding regional variables and the early integration into the wine industry of computers, as well as the beginning of geo-referenced data analysis using rule-based expert systems (ES). Data from geographic data analysis, particularly in agriculture, horticulture, and viticulture, has to be more reliable[17]**.**The authors drew from a variety of sources and used cutting-edge computational methods to their work. The scientists used state-of-the-art technical tools to incorporate previous scientific approaches for data gathering, transport, and analysis utilising artificial neural and fuzzy networks [18].

Drought Projections

Use of recurrent neural networks (RNNs) has been used in this study to investigate the association between El Nino-induced long-term dryness and wetness (RNNs). With the comparison of the Palmer Z Index (PZI) from 1998 to 1999 El Nio, the long-term projection of El Nio bringing enough rainfall is confirmed. Precipitation forecasts were put to the test using RNN, which discovered a statistically significant correlation between what was anticipated and what really happened[19].

Frost Control in Greenhouses

This article discusses the use of ANN to keep greenhouse frost at bay. Frost is a really difficult technological problem. Greenhouse production benefits from thermal comfort, which requires a significant amount of heating and ventilation energy. A Levenberg-Marquardt back propagation approach was used to train a Multi-Layer Perceptron ANN to prevent frost in greenhouses in the Mexico area. Forecasts for both summer and winter temperatures are made by this sophisticated frost control system [20].

Prediction of Soil Texture

The FFNN model, an “artificial neural network technique”, is used to predict soil texture in this contribution. Using remote sensing data and the “Self-Organizing Map (SOM)”, soil samples were analysed for the amount of sand, silt, and clay. The neural network model was built using data gathered from field observations. According to the results of an experiment, the formula for FFNN's success is sound [21]. The authors built a “cloud-based cultivation management system” to let farmers look at their crops from anywhere utilising the dataset. It was decided to install the suggested monitoring system's hardware module's sensors, gadgets, and integrated circuits (ICs) in the field. With a mobile phone as a remote control, a cloud-based software source was employed. Voltage signals having a

threshold value of a predetermined threshold would be used in the proposed system to assist determine soil moisture content [22].

Remote Sensing Scene Classification

With regard to remote sensing datasets, this article discusses the use of CNN (Convolution Neural Networks). Like other neural networks, CNN should be trained, fine-tuned, and applied to remote sensing data. For the trials, the authors employed 3 different data sets of RS with 6 different and common data sets of CNN, and then compared the outcomes. In addition, the work discusses an "information technology-based crop disease prediction system" that warns farmers about the illness damaging crops and the preventive and treatment to be done for data analysis using mathematical modelling. Pests may be identified with the use of computer technology. The semi-supervised learning method was found to be effective by the authors [23], [24].

Forecasting Agricultural Production

The goal of the study was to examine how IT may be used to improve agricultural output estimates. Neural networks outperform other approaches, such as ARMA and B-J, in their ability to predict future events. While BP net is effective at learning, GRNN has a high prediction rate and can perform well even with minimal data, but it was not ideal for nonlinear and multivariable models, which are more difficult to predict [25]. GRNN has a high convergence rate and strong prediction ability [26].

Pistachio Nuts Classification

The price of pistachios depends on the quality of the crop. Closed-head pistachios are a key commodity for the economy, export, and marketing, thus it is essential to evaluate their quality [27], [28]. An artificial neural network (ANN) classification method for pistachio nuts is discussed in this article. Farming pistachios is a profitable endeavour. This device's ANN was trained by analysing acoustic signals generated when a pistachio is pressed on a steel plate. The 'Principle Component Analysis (PCA)' technique is used to reduce the size of the signal. Different types of ANN were utilised to compare the experimental findings, and the suggested technique yielded a 99.8% accuracy rate. 75 percent of the received signal was utilised for network training, while 25 percent was used for testing using a "multilayer with hyperbolic tangent sigmoid transfer"[29].

Disease recognition in plants

According to the paper, a deep convolution neural network may be used to identify maize leaf disease in real time. Automatic diagnosis of maize illnesses and the prevention of large crop losses are now achievable with the newest access to smart devices. On a GPU system, performance of deep neural networks may be improved by altering hyper-parameters and pooling combinations. Additionally, the model's parameters are tweaked such that it may be used for real-time inference. The pre-trained CNN model was evaluated on a Raspberry Pi 3 with an Intel Movidius Neural Compute Stick, which has specific CNN hardware blocks, for the identification of maize leaf diseases [30]. It was suggested that an ANN-based ongoing classification approach may help solve a wide range of agricultural classification problems[31]. Convolution neural networks (CNN) and generative adversarial networks (GEN) form the basis of this memory-based approach (GAN). Crop pest detection and categorization of plant leaves were used in the assessment of this approach. The strategy was also put to the test against the conventional CNN methodology.. The findings reveal that the normal CNNs correctly categorise the categories for single task difficulties, but they are prone to forgetting problems and are unable to balance new and old tasks in continuous tasks. The authors' approach has the potential to help people retain information and prevent it from being forgotten. Because of this, it is better able to discriminate between old and new duties [32]. When it came to classifying leaf illness, the researchers in this study opted for a CNN-based model known as the Mobile Net. On the dataset, a number of experiments were run in order to get reliable results. In comparison to the prior approaches, the suggested system is guaranteed to provide more accurate results [33]. Image processing and deep learning were used by the researchers [34] to identify the illness in its earliest stages. A Convolution Neural Network was utilised to categorise the many illnesses that impact plants using Keras' hidden layer method [35]. Using the CNN model, potato leaves may be categorised into three groups: "healthy leaves, early blight and late blight sick leaves"[36]. Plant diseases are detected using a deep

learning Google Net architecture. There are 54,306 photos of 14 crops and their illnesses in a public database that the model is trained on [37]. To take pictures of plant leaves, a mobile app has been created for both Android and iOS devices. Using a web service, the CNN model is used to make a diagnosis[38], [39]. Using photographs of sick plants, disease agents may be identified and isolated. Convolutional Neural Networks' classification capabilities are used to provide consistently accurate results. Google's 'Inception v3' pre-trained model is employed. The 'Plant Village Dataset' is used to train the Inception v3 model on a collection of sick plants. F1 score, precision, and recall are used to assess the created detection method [40].Pre-processing, Training, and Identification are the tools needed to accurately identify and characterise the disease in leaf photos[41]. Leaf photos may be analysed using a CNN technique to identify the presence of disease [42]. Leaf image categorization is used in the paper's objective of creating a disease recognition model. They use image processing with a CNN to identify plant illnesses (CNN) [43]. In their study, researchers looked at three basic Neural Network architectures: SS- Detector, R-CNN and Faster R-CNN. It is possible to detect a wide range of diseases using the system proposed in the study. It also has the capacity to handle complicated situations [44]. The DCNN architecture presented in the article uses two branches of skip connections to identify plant illnesses. Compared to ResNet, AlexNet, Mobilenet, Xception, and VGGNet, they found it to be the best among the DCNNs. Even with a lesser number of parameters and a quicker training period, our technique consistently outperforms the reference methods [45].

Using the CNN technique to extract information from input photos, the researchers were able to distinguish between damaged and healthy leaves on different plants. These derived characteristics aid in the classification of pictures in datasets into the most appropriate class. They found that the suggested system takes an average of 3.8 seconds to accurately identify the picture class with a precision of over 94.5% [46]. Based on three pre-trained models, such as VGG-16, ResNet-50, and ResNet-50 v2, the proposed framework primarily focused on the transfer learning phenomena and evaluated the 3-models based on “transfer learning models” using various standard evaluation criteria. Accuracy rates of 98.74 percent for the VGG-16 transfer learning model and 98.84 percent and 98.21 percent for the ResNet-50 and ResNet-50v2 based transfer learning models were attained [47]. In order to detect the diseased leaf of the betel vine, a colour study was carried out. An important step in cropping removes unnecessary information that was obtained during pre-processing. This feature's primary function is to distinguish a rotting leaf from an otherwise healthy one. After that, the picture is converted to RGB, HSV, and YCbCr colour spaces, as appropriate. According to the findings, the HSV was the most accurate in identifying the rotten plant tissue among the other tests. White pixels are used to estimate how much of the decaying component has been removed [48]. Identification of disease-causing variables in plant crops is aided by artificial neural networks (ANNs). The plant's picture is transformed from RGB to grayscale in this method. Colour characteristics and histograms are derived from this model's template. Colour characteristics such as the mean and SD are covered in this study. The disease-infected banana plant's feedback propagations NN is identified using these characteristics [49]. The use of ANN and other remote sensing technologies has exploded in recent years. Remote sensing data was utilised to predict the age of coniferous forests using statistical and artificial neural network (ANN) techniques. To estimate forest stock amount, remote sensing images and GIS were integrated with neural networks (ANNs) [50]. A BP neural network was used to develop a RS-based “forest biomass nonlinear model system” for Wangqing, Jilin Province's natural wooded areas[51]. The researchers used ANNs in conjunction with GIS to create an ecotourism suitability evaluation model for the area. It is possible to use this model as a benchmark for evaluating domestic ecotourism operations[52]. The spatial shift in soil organic carbon was simulated using an ANN model. The ANN model's accuracy was tested by comparing the correlation between soil organic carbon content measurements and the ANN model's change value. According to most statistical approaches, R values ranged from 0.50 to 0.61, however the R values in this study were larger than 0.7 [53]. The ANN model's "black box" premise was described as being very accurate[54]. Table 1 shows the different techniques of machine learning uses in different plant or crops with performance accuracy in agricultural ecosystem.

CONCLUSION

When it comes to modelling in agriculture, "machine learning (ML) algorithms" have emerged as a viable alternative and complementary technique. These days, agricultural production, yield prediction, and forest management studies are increasingly using machine learning algorithms. An application of artificial intelligence known as machine learning is one that allows a system to learn from examples and experience without the need for any explicit programming. Machine learning is a class of methods that enables research-relevant systems to be better predicted by software applications. When developing algorithms for data input and output prediction, ML relies on statistical analysis to keep outputs up-to-date as new data becomes available. There are several major applications of machine learning in agriculture ecosystem such as Drip Irrigation System, Viticulture, Drought Projections, Frost Control in Greenhouses, Prediction of Soil Texture, Remote Sensing Scene Classification, Forecasting Agricultural Production, Disease recognition in plants, etc.

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Table 1: Machine learning application in agriculture

Plants/ Crops	Samples/ Datasets	Objectives	Model Designs	Performances	Year	Refs
Tomato	67 images	to improve the accuracy of the ResNet model	ResNet CNN	disease identification accuracy of the transfer learning model is 83.75%	2020	[55]
13 different plants	1300 images	To keep the farmed crop at a minimum risk of destruction.	CNN	94.8% accuracy		[56]
Banana, beans, jackfruit, lemon, mango, potato	106 images	an image segmentation system designed to identify and classify plant leaf diseases automatically	ANN,Bayes classifier, Fuzzy Logic and hybrid algorithms	97.6% accuracy	2017	[24]
Different crops	500 images	Preventive measures may be implemented to increase output by identifying and preventing plant diseases in their earliest stages,	Multilayer CNN	97% accuracy	2020	[23]
Potato, Pepper and parameters for training and test.	5000 images	Image processing as well as deep learning techniques may be used to identify plant diseases in their earliest stages.	CNN	95.8% accuracy	2021	[57]
Rice Plant	9857 images	Diagnose and treat rice plant problems in a timely and accurate manner	RDA-CNN	96.595%	2021	[58]
maize crop	5939 images	to recognize diseased and healthy maize crop leaves in digital photos automatically	CNN	overall classification accuracy of 95.99% with average recall of 95.96% on the separate test dataset	2022	[59]
Groundnut	6400 images	in order to combat a variety of pests in groundnut production	DCNN	99.88% accuracy	2020	[60]

Vigna Mungo	433 leaves images of Vigna Mungo	Real-time detection of illness using an automated system developed from scratch.	CNN	91.234% for VirLeafNet-1 96.429% for VirLeafNet-2 97.403% for VirLeafNet-3	2020	[61]
Grape	Plant village dataset	Applying Feature Reduction to the "Hybrid Convolutional Neural Network" for Leaf Disease Recognition	Hybrid CNN	AUC of 98.7%	2022	[62]

Figure 1: Agriculture ecosystem

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RESEARCH ARTICLE

Salinity Induced Changes in Growth and Biochemical Constituents of *Vigna mungo* L. (Co Gg 912).

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ABSTRACT

The present study was made to the effect of different concentrations of sodium chloride (NaCl) on germination and biochemical constituents of *Vigna mungo* L. CoGg 912. The germination studies were conducted with various concentrations of NaCl (0, 10, 25, 50, 75, 100, 125, 150mM) and the data were assessed 15th day after germination. The percentage shoot length, root length, fresh and dry weight, leaf area increased up to optimum level of 25mM and thereafter all the parameters drastically reduced. The photosynthetic pigment, carotenoid, protein and starch content increased up to 25mM level and at higher concentrations reduced gradually. The amino acid and sugar content decreased up to 25mM level and there after gradually increased. The accumulation of proline and glycinebetaine content increased with increasing concentration at extreme level of 150mM NaCl.

Keywords: Salinity, Germination, Osmolytes, vigour index, *V. mungo*, Phytotoxicity.

INTRODUCTION

Soil salinity is one of the most damaging abiotic stresses that limit agricultural productivity [1]. Salinity is one of the most serious abiotic stresses limiting plant growth and development, especially in salt-sensitive crops. The detrimental effect of high salinity on plants can be observed at the wholes-plant level as the death of the plant and/ or decreases in productivity. Many plants develop mechanisms either to exclude salt from their cells or to tolerate its presence within the cells. During the onset and development of salt stress within a plant, all major processes such as photosynthesis, protein synthesis and energy and lipid metabolism are affected [2]. In relation to seedling growth, the cotyledons and the embryonic axes were supressed by NaCl. They were smaller than in distilled water because of reduced fresh weight resulting from reduced water absorption [3]. Salinity have the negative impact on shoot and

root length, fresh and dry weight. There were significant reduction of root lengths, root fresh and dry weight, shoot length, shoot fresh and dry weight in all genotypes under saline condition [4] and *Vigna mungo* [5], *Vigna radiate* [6], *Vignaaconitifolia* [7].Production of economic yield is determined by the proportion of assimilates partitioned to reproductive organ. Thus, it is important to understand the dry matter accumulation pattern for the improvement of yield level in saline soils. The effect of salinity on the pattern of dry matter accumulation and the partitioning in leaves, stem, reproductive organ and roots [4]. The content of leaf chlorophyll is a key indicator of photosynthetic potential and in association with leaf area index it has been found to be a major indicator of crop productivity [8]**.** The chlorophyll content was sharply decreased at higher NaCl concentration in *Solanum melongiana* [2]; *Oryza sativa* [9] and sorghum [10]. The amino acids content was decreased progressively in shoots and roots of maize and broad bean plants with increasing NaCl stress [11]. [12], reported that amino acids play the role of osmoprotectants by decreasing osmotic potential and accumulation under salinity stress. Total free amino acids in the leaves are reported to be higher tolerant than in salt sensitive lines of Phragmites [13]; *Nicotiana tabacum* [14] and certain crop species like *Vigna mungo* [15].

Protein content in the tissue of many plants declined under drought or salinity stress, because of proteolysis and decreased protein synthesis. It is possible that proline accumulation contributes to the osmotic adjustment at the cellular level [16]. Salt stress increases total soluble protein contents in mung bean varieties under both salt stress and non-salt stress [17]. The soluble protein contents in shoots and roots of maize plants were appreciably lowered by salinity stress. however, the pattern of this reduction was found to be constant at all salinity levels [18]; [19]; [11].Among the widely distributed osmolytes, proline is the most fundamental one and is predominantly found in higher plants. Being an amino acid and characteristically rigid, it performs vital roles in response to various kinds of abiotic stress through adaptation, signalling, and recovery mechanisms. Proline is found to accumulate in the chloroplast and cytoplasm and when plants are exposed to stress considerably accumulation of proline occurs in order to induce stress tolerance [20]. Glycinebetaine contents significantly increased under salt stress in mung bean varieties [17]. The quarternary ammonium compound GB (N,N,N-trimethylglycine), also referred to as original betaine, is a methylated glycine derivative. Among the betaines, GB is the most abundant in plants and was excessively produced in response to dehydration caused by different abiotic stresses like drought, salinity and extreme temperature [21]; [22]. Glycinebetaine is one of the main compatible compounds present in Poaceae and Chenopodiaceae under salinity, and which is also involved in many other protective mechanisms against stress-related plant disorders [23]; [24]; [25].

MATERIALS AND METHODS

Germination Studies

During September 2021, the *Vigna mungo* seeds were collected from Seed Research Institute, Aduthurai, Thanjavur district, Tamil Nadu. The seeds were surface sterilized for two minutes in 0.2% mercuric chloride ($HgCl_2$) solution. The surface sterilized seeds were thoroughly washed with tap water and then followed by distilled water and then the seeds were arranged equi-specially on the periphery of sterilized Petri dish lined with filter paper. Each Petri plate were allowed for 10 seeds and treated with various concentration of sodium chloride ranging from 10-150 mM NaCl. The control seeds were treated with distilled water. The Petri dish were kept diffused at room temperature (30^0C). the number of seeds germinated in each treatment was counted daily up to 15^{th} day after sowing. The germination percentage were calculated on 15^{th} day after sowing. Five seedling of each replicate was selected for recording the morphological parameters such as shoot length, root length, fresh and dry weight and leaf area.

Germination percentage

After 48 hours, the seeds were examined to determine the germination percentage. Germination was assumed to be completed when the radicle pierced through the seed coat (Mayer and Mayber, 1982).

Morphological studies

Five seedlings were removed carefully from each and every plastic tray on the fifteenth day and washed in water for studying the morphological parameters. The length of root and shoot was measured with the help of a scale. Total leaf area was calculated by measuring the length, width and number of leaves and multiplied by correlation factor (0.66) derived from the method of Yoshida *et al.*, 1972.

Fresh weight and dry weight

The seedlings were removed from plastic trays and washed thoroughly. For the estimation of fresh weight of leaf, stem and root portions were separated and weighed. The seedlings were removed from plastic trays and washed thoroughly. They were separated into root and shoot and they were kept in the hot air oven at 80°C for 24 hours. The dry weight of the seedlings was taken by using a single pan electrical balance.

Biochemical studies

Germinated seedlings of black gram on 15th day were separated into root and shoot and they were used for biochemical analyses such as chlorophyll, carotenoid, starch, amino acids, protein and sugars by using the following methods. The chlorophyll content was estimated according to the protocol mentioned by Arnon's(1949).The estimation of carotenoid content was carried out according to the protocol mentioned by Davis(1965).The estimation of amino acids content was carried out according to the protocol mentioned by Moore and Stein(1948).The protein content was estimated according to the protocol of Lowry *et al.*, 1951.The estimation of sugar content was carried out according to the protocol mentioned by Nelson(1944).The estimation of starch was carried out according to the protocol given by Sumner and Somers(1949).Proline was extracted and estimated according to the method of Bates *et al.*, 1973. The samples were extracted and estimated according to the method of Grieve and Grattan (1983).The data were statistically analysed by the method of complete randomized block design (ANOVA one-way method).The F value were significant at both 1% and 5% level.

RESULT AND DISCUSSION

The present investigation deals with the effect of different concentration of sodium chloride on the seed germination and biochemical constituents of black gram (*Vigna mungo L.*, Co Gg 912).

Germination percentage

The germination percentage of *Vigna mungo*as affected by different concentration of sodium chloride. The germination percentage was found to be maximum at control around 95% and also the optimum level 25mM around 75% on 15th day after sowing. The minimum percentage of germination was recorded at 150 mM NaCl and this was 15% higher when compare to control. The seeds showed emergence of radicles only on third day after sowing and the maximum emergence was only up to 25mM, on the 5th day after sowing the germination was observed in all the concentrations. The experimental samples collected on the 15th day after treatment. Germination and seedling stages are critical life stages for plant survival and appropriate seedling establishment, particularly under stress conditions. The findings of this study indicated that seeds germination and establishment of black gram seedlings were inhibited gradually by increasing salinity stress. At a high salinity level of 150mM NaCl, seed germination was completely inhibited. In this respect, many studies reported that increasing salinity level decreased germination percentage and germination speed in field pea [36], wheat [37] and other legumes [38]; [39]; [40]. In our study, although germination percentage was significantly affected, the analysis revealed the existence of considerable genetic variation for seed germination potential under salt stress conditions, with varieties Neoplanta and Adonai being classified as most tolerant at low and medium stress levels. At high stress level, all genotypes suffered significant decrease in germination rate, with the exception of Neoplanta which retained a high germination ability [41].

Fresh weight and dry weight

The data on effect of sodium chloride on the fresh weight of shoot and root of *Vigna mungo*are presented in Table. The fresh weight of shoot always higher than the root. The highest increase in the fresh weight of shoot and root was observed up to 25mM and this was 28.77%, 90.90% increase over that of control on 15th day after sowing. Beyond this optimum level the fresh weight sharply declined. The F value were significant at both 1% and 5% level. The results on the effect of sodium chloride on the dry weight of shoot and root of *Vigna mungo* are presented in table. The dry weight of shoot and root organ increase with increasing in salinity up to 25mM and this could be 128.57%, 200% higher when compared to control. At higher concentration the dry weight drastically reduced. The F value were significant at both 1% and 5% level. Apart from the classification of genotypes according to their response to salt stress, this study underlines the possibility of using this approach for screening for salt tolerance at early growth stages, employing high stress levels and preferably combining them with germination percentage and data for root and shoot length. In this regard, root length reduction in response to salt stress has been previously proposed as an indicator of salinity tolerance at germination stage in soybean [41]. The similar results are coincide with our research work. From the result of this study, it is evident that the toxicity in the salinity treatments is expressed more clearly in dry weight. This finding supports early findings which indicated that growth inhibition by NaCl treatments was greater for dry biomass production [42]; [43]; [40]. The fresh weight and dry weight of shoot and root of black gram genotypes was decreased remarkably under saline stress [44].

Leaf area

The sodium chloride salinity had increased the leaf area with increasing concentration and was recorded at 71.78% higher up to optimum when compared to control. Beyond these optimum concentrations the leaf area was reduced gradually.

Chlorophyll

Sodium chloride treatment had stimulated the chlorophyll content (Fig- 1)up to 25mM and there after it was steadily declined. The highest accumulation of total chlorophyll synthesis was observed at 25mM and this was 85.17% increase over control on 15th day after sowing. The chlorophyll-a was always higher than the chlorophyll b for all the concentration. The lower concentration increased the chlorophyll pigments and at higher level reduced the chlorophyll. The photosynthesis pigments, including chl a, chl b, Tot. 'chl' , significantly reduced by moderate and high salinity treatments observed in two variety of pistachio. Several studies suggest chlorophyll content as a biochemical marker of salt tolerance in plants. It is known that salt tolerant plants show increased or unchanged chlorophyll levels under salinity conditions whereas chlorophyll contents decreased in salt-sensitive plants [45]; [46]; [47]. A decline in Chl content and fluorescence causes a significant reduction in photosynthetic function when plants respond to salt stress in black gram [48].The sodium chloride salinity had stimulated the carotenoid synthesize up to 25mM and this was observed at 66.25% higher when compared to control (Fig-1). At higher concentration the carotenoid content reduced drastically.

Protein

The results on the effect of different concentration of NaCl on leaf, stem and root of *Vigna mungo* are given in Fig-3. Sodium chloride salinity increased the protein content of leaf, stem and root up to 25mM and this was found to be 157.71%, 40% and 52.07% higher when compared to control on 15th day respectively. In contrast to [49], who noticed an increase in the total protein under high saline conditions. However, the same results were documented in [50] study, who noticed the rise of total protein content increased under saline stress, but this content decreased under high levels of salt, which agreed with our study. Total protein consists of many types of protein, including soluble types. The plant might tolerate saline stress at a specific level by transforming more soluble protein from total protein [51].

Amino acid

The sodium chloride salinity had decreased the amino acid content of leaf, stem and root up to 25mM NaCl and beyond this level an increasing trend was noticed on both the sampling days. The maximum decrease in amino acid content was observed at 25mM and this was 51.42%, 31.42%, and 54.62% less when compared to control on15th day after salt treatment (Fig 4).In [52] documented an increase in the level of free amino acids in wheat leaf plants under salinity control. In [53] reported similar findings. In [54] mentioned that the reason behind the increase in free amino acid content in tomatoes (*Solanum lycopersicum*) treated with saline water was protein hydrolysis. Some reporters explained that the main reason for increasing free amino acid under saline conditions is that plants utilise free amino acid as a substitutional substance in mitochondrial respiration as carbohydrate and chlorophyll decrease [55]; [56]; [51].

Total sugar

The data on the effect of NaCl salinity on the total sugar content of leaf, stem and root of *Vigna mungo* are given in Fig-5. The total sugar content decreased with increasing concentration up to 25mM, beyond this level there was gradual increase in the total sugar content in all the three tissues. The leaf had more total sugar than the stem and root. The optimum level of 25mM NaCl decreased the total sugar content and this could be8.71%, 35.25%and 22.5% on 15th day respectively (Fig-5).The salt treatments induced noticeable variations in the soluble sugar contents in both parts of each of pistachio cultivars under investigation. The accumulation of soluble sugars in response to salinity was observed in leaves and roots of BZ. It is believed that under salinity stress allocation of assimilates for osmotic homeostasis as well as partitioning into roots along with other compatible solutes contribute to osmotic adjustment [56]; [57].

Proline

The data on the effect of NaCl salinity on the proline content of leaf, stem and root of *Vigna mungo* are given in Fig-6. There was considerably increase in the accumulation of proline with increasing salinity up to extreme level of 150mM and this could be 85.19%, 78.18% and 94.62% higher when compare to control on 15th day respectively. Under salinity stress plants accumulate compatible solutes such as proline and soluble sugars which are known for their osmoprotection activity [58]. The accumulation of metabolites that act as compatible solutes is one of the common responses of plants to changes in the external osmotic potential [59].

Glycinebetaine

The data on the effect of NaCl salinity on the glycinebetaine content of leaf, stem and root of *Vigna mungo* are given in Fig-7. There was considerably increase in the accumulation of glycinebetaine with increasing salinity up to 150mM and this could be 129.26%, 110.75% and 98.09% higher when compare to control on 15th day respectively. GB and proline levels are highly correlated under salinity conditions, and their sum is equal in young expanding tissues of both high- and low-nitrogen grown plants. The presence of interchangeable levels of both compounds in young tissues, independent of nitrogen nutrition, would imply that resources are allocated in growing tissues in order to support and protect young growing tissues [60]; [25].

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Table 1. Effect of NaCl on growth parameter of *Vigna mungo* L. (Co Gg 912) on 15 DAS. Values are mean and Standard Error of five replicates.

Concentration (mM)	Growth Parameter (cm plant^{-1})		Fresh Weight (g plant^{-1})		Dry Weight (g plant^{-1})		Leaf Area (cm^2 plant^{-1})
	Shoot Length	Root Length	Shoot	Root	Shoot	Root	
0	12±1.555	4±0.289	0.73±0.035	0.11±0.012	0.14±0.012	0.04±0.006	0.84±0.017
10	18±1.443	6±1.155	0.82±0.029	0.15±0.017	0.21±0.023	0.06±0.012	1.265±0.014
25	20±0.866	8±0.866	0.94±0.029	0.21±0.023	0.32±0.029	0.12±0.012	1.443±0.019
50	19±1.155	7±1.155	0.73±0.046	0.18±0.012	0.27±0.023	0.09±0.012	0.862±0.013
75	17±0.866	6±0.866	0.61±0.035	0.14±0.017	0.2±0.017	0.07±0.006	0.681±0.012
100	15±1.443	5±0.577	0.55±0.023	0.12±0.017	0.15±0.017	0.06±0.006	0.652±0.016
125	12±0.577	4±0.289	0.48±0.035	0.1±0.012	0.1±0.006	0.05±0.006	0.565±0.014
150	10±1.155	3±0.289	0.32±0.04	0.07±0.003	0.07±0.012	0.03±0.006	0.47±0.017
F	10.957*	4.782*	32.87*	9.073*	20.388*	11.698*	483.834**

F values difference between treatment and number of days.* The F values were significant at 1% level. ** The F values were significant at 5% level

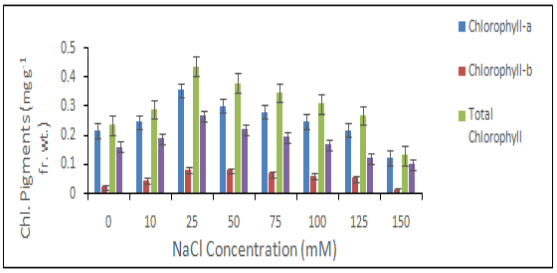

Figure 1. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM)on chlorophyll pigments (mgg^{-1}fr. wt.) of *Vigna mungo* L. at 15 DAS. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

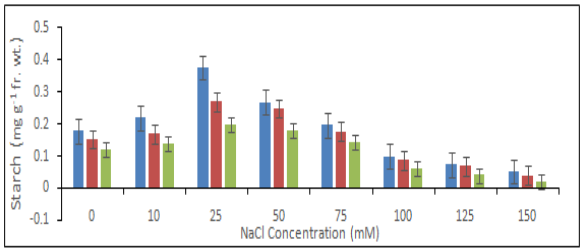

Figure 2. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) on starch (mgg^{-1}fr. wt.) of *Vigna mungo* L. at 15 DAS. Values are mean and standard error of five replicate. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

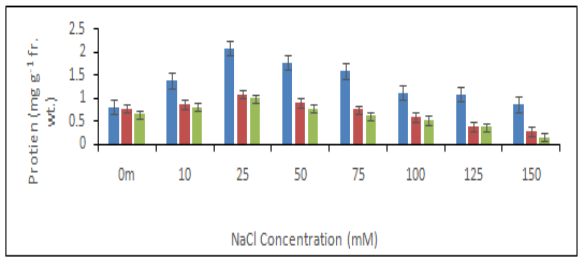

Figure 3. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) on protein (mgg^{-1}fr. wt.) of *Vigna mungo* L. at 15 DAS. Values are mean and standard error of five replicate. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

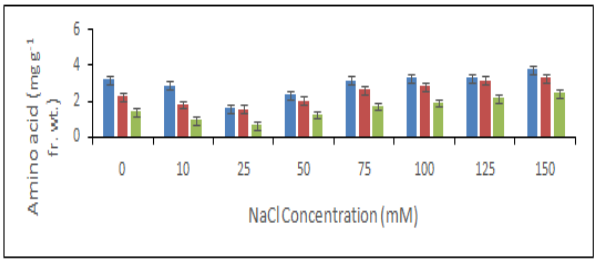

Figure 4. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) on amino acid content (mgg^{-1}fr. wt.) of *Vigna mungo* L. at 15 DAS. Values are mean and standard error of five replicate. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

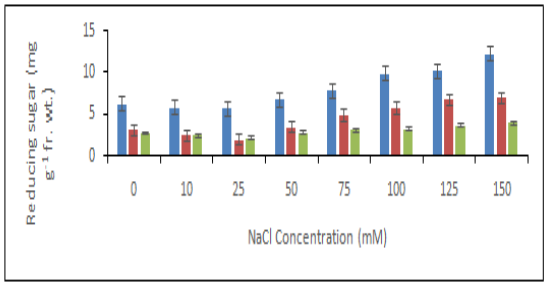

Figure 5. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) on reducing sugar(mgg^{-1}fr. wt.) of *Vigna mungo* L. at 15 DAS. Values are mean and standard error of five replicate. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

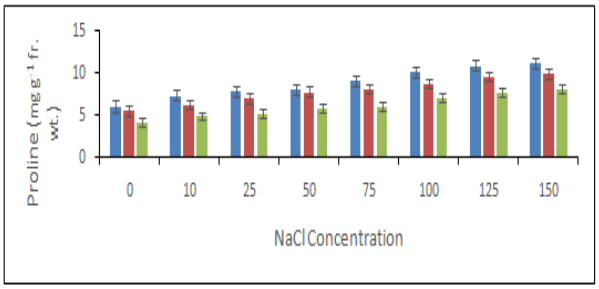

Figure 6. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) on proline (mgg^{-1}fr. wt.) of *Vigna mungo* at 15 DAS. Values are mean and Standard Error of five replicate. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

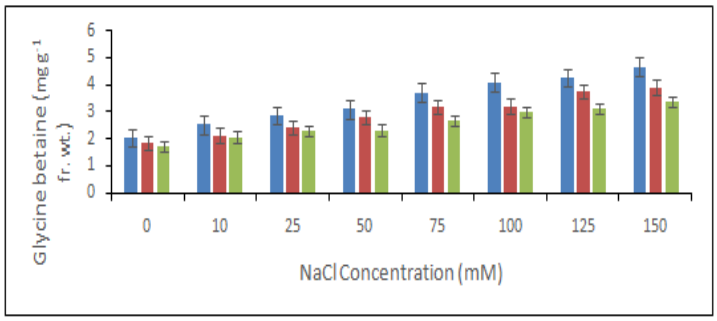

Figure 7. Effect of different NaCl concentrations (10mM, 25mM, 50mM, 75mM, 100mM, 125mM and 150mM) on glycinebetaine (mgg^{-1}fr. wt.) of *Vigna mungo* at 15 DAS. Values are mean and Standard Error of five replicate. Values represented in Bars are mean of three replicates (n=3) and (±) standard error.

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RESEARCH ARTICLE

Smart Pill Delivery System for Alzheimer Patients

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ABSTRACT

The majority of peoples have memory problems but are not impacted by Alzheimer's disease, whereas all Alzheimer's patients have memory problems. As a result, they've become disoriented and have lost track of their daily routine. Many scientists and researchers are aiming to solve the abovementioned dilemma. This system is designed to assist patients, primarily seniors, in taking their medications on time and in an easy manner without the chance of missing pills, in addition to lowering the changes of accidently overdosing or under dosing. Failure to take medications as prescribed might result in complications such as delayed recovery, sickness, and even death. It assists Alzheimer's patients by informing and alerting them to the proper dose at the appropriate time. It supports Alzheimer's sufferers in distributing medications by indicating the prescription time as well as when the tablet is taken, such as before or after food. The tablet will also have a voice alert feature. Patients' caretakers were contacted if they did not take their tablets on time using the GSM module's calling process.

Keywords: dilemma–Issues/Problem, neurodegenerative disorder –Neurological Disease.

INTRODUCTION

Alzheimer's disease is a neurodegenerative that affects millions of people worldwide. Alzheimer's disease and other types of dementia have a significant influence not just on persons who are diagnosed, butalso on their families and society as a whole. Various studies and organizations have presented technologies to assist in the relief of their people. Products that claim to enhance patient safety yet fail to effectively protect patient personal information as a result, we created a smart pill delivery system for Alzheimer's patients that will help them maintain their regular medication intake.

EXISTING WORK

The flowchart describes the system's implementation, operations and functionality as well as its features. The SPE system has a built-in fingerprint scanner. Consider a patient who needs to take medications in the morning and afternoon using an application interface and an in-built calendar, a doctor or the person in control can arrange appointments. At the appointed time, an alarm will ring, reminding the user to take their medicine. The authorized user then places their finger on the finger print scanner. After that, the fingerprint will be compared to a data base that has a preset of information. The cup carrying the drugs for that period will be distributed if the fingerprints match. If the user forgets to take their pills, notification and alarms will be sent to both user's phone and their loved ones. This system is to provide at the right time to take drug for the patient. The main feature of the SPE system is that accommodate multiple users at once while also managing and monitoring actions to prevent errors. A powerful user authentication and authorization security mechanism is provided by the suggested system. In Existing Methodology, finger prints were used, which make it complexity to take pills. Alzheimer's people didn't know which tablet they took because it wasn't specified in the existing system.

METHODS AND TECHNIQUES IMPLIED

Both hardware and software design principles are covered. It contains the Arduino Mega, which is the brains of the system and controls its overall operation. In the proposed methodology, tablets are supplied topatients at the appropriate timing through voice alarm. There was also an LCD display that showed the name of the Tablet as well as the amount of time it had been used.

ALGORITHM

STEP 1: Start
STEP 2: Given time input is compared with the Real Time Clock (RTC) if it returns **TRUE** goes to step3 else step2
STEP 3: Voice alert given as remainder
STEP 4: If press Touch sensor and take pill goes step 2else step 5
STEP 5: Calling process is enabled.
STEP 6: Stop

FUNCTION OF SPDS

To begin, the system scans the EEPROM data and fills it if it is missing. If the time and date are not provided, the system will set them, and if the audio file is not available, the system will insert it through the SD card. The first alarm time, second alarm time, third alarm time, and fourth alarm time are displayedon the display unit, followed by the current time and date. Once the alarm time and current time are matched, the voice controller is activated, and the medicine name and medication time (before or after food) are displayed on the LCD display. Patient should press the touch sensor and swallow a medication at that time. If the patient does not press the touch sensor, the system enters the calling process after the five voice alert reminders, following which the system calls to caretaker and then system waits for the next time interval. Patients who want to halt the calling process should hold the touch sensor for twenty seconds, after which the calling process will be stopped and the system will wait for the next time interval and soon.

HARDWARE DESCRIPTION

Arduino Mega

Arduino mega 2560 is heart of the smart pill delivery system. It only connected with each component.

RTC Module

In pill delivery system, the Real Time Clock were used it shows current time and date. When the current time matched to medication prescribed time the system get activate i.e., LCD display and voice alert is enable.

LCD Display

Liquid crystal displays (LCDs) are flat panel displays that use liquid crystals to operate. It has two rows and two columns. The smart pill dispenser displays "Alzheimer's loading..." before displaying the current time and date. The first alarm time, second alarm time, third alarm time, and fourth alarm time are all displayed on the smart pill distribution system. At the specified time, the name of the medicine/pill and when it should be taken, such as before or after eating, are displayed. The display device displays the current time and sets the alarm time to be two minutes apart when the system is in demo mode.

Touch Sensor

Touch sensors detect and record physical contact or embrace on their surface. Smart pill delivery system use capacitive touch sensor to transform analogue signals to digital signals. At medication time the patient should take a pill by pressing the touch sensor. If patients didn't press touch sensor the system reminds to take a pill continuously and it goes calling process when the timeout.

Audio Amplifier

An audio amplifier's function is to reproduce audio signals at the necessary volume and power levels at sound-producing output devices. This audio amplifier reproduces the smart pill delivery system's vocal tones.

DF Mini Player

The DF Mini player is a compact, low-cost MP3 player with a simple speaker output. It comes with a voice memo SD card. Smart pill delivery system has voice comments which present in SD card.

CONNECTIONS OF SMART PILL DELIVERY SYSTEM

The required hardware in the form of arduino mega connected with GSM module, RTC module, touchsensor, LCD display unit and audio amplifier. GSM module transmitter pin connected with arduino mega receiver pin (pin19) and GSM module receiver pin connected with arduino mega transmitter pin (pin18). LCD display serial clock (SCL) pin were connected to both RTC module serial data and arduino mega serial clock pin (pin21), LCD display serial data (SDA) pin were connected to both RTC module serial clock and arduino mega serial data pin (pin20). Audio amplifier (PAM amp) is connected with arduino at pin2 through DF mini player. Touch sensor output is connected to arduino at pin 12 and touch sensor ground pin connected to 10K resistor.

SIMULATION IN PROTEUS DESIGN SUITE

Proteus Design Suite is a software tool which is used for simulation the circuits or designing the hardware. The Proteus Design Suite was used to simulate the smart pill delivery system which includes an Arduino Mega, GSM module, RTC module, LCD display and Audio amplifier. The Proteus Design Suite is brought smart pill delivery system hardware together and give simulated result. The smart pill delivery system is made by PCB board and it simulation is done by Proteus Design Suite.

RESULT

In Smart Pill Delivery System have two output devices which are LCD display and Speaker. Speaker speaks displayed words at given time. LCD display shows current time, date, pill name and pill taken time such as pre-food or post-food.

CONCLUSION

The proposed method is designed to be simple and beneficial for elderly patients, particularly those who forget to take their medications on time. It has the ability to send any leftovers. In the event that thepatient does not take his or

her meds after a reasonable amount of time has passed, the system sends a signal as a call to the caretaker's phone number specified in the software, which is then dumped into the Arduino Mega. As the world moves toward digitization, this kit can be expanded into a complete embedded system based solution that can be used to track the health of and body, anywhere, at any time and can be built for hospitals on a big scale to assist nurses in keeping track of many patients prescriptions.

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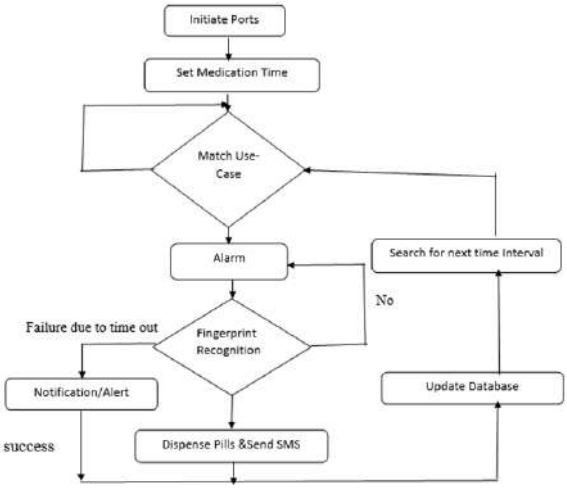

Figure 1: Flow Chart of Existing Work

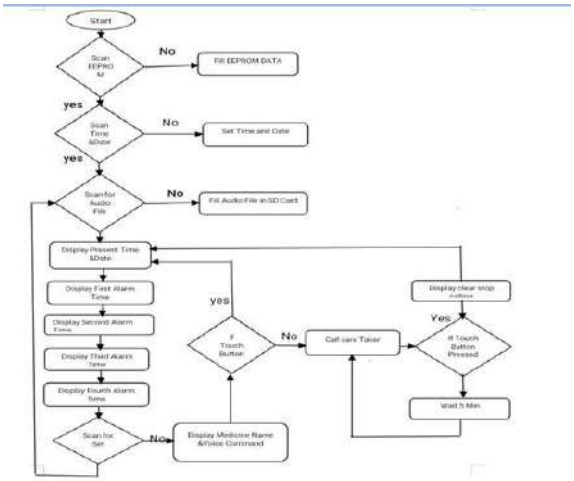

Figure 2: Flow Chart of SPDS

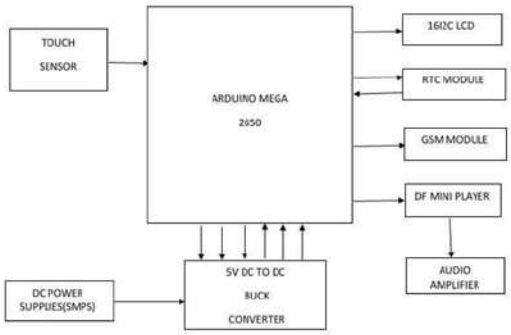

Figure 3: Block Diagram of SPDS

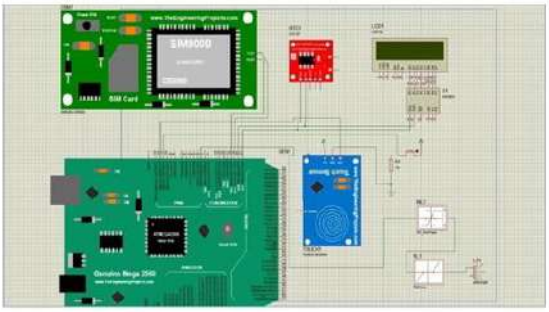

Figure 4: Simulation Diagram

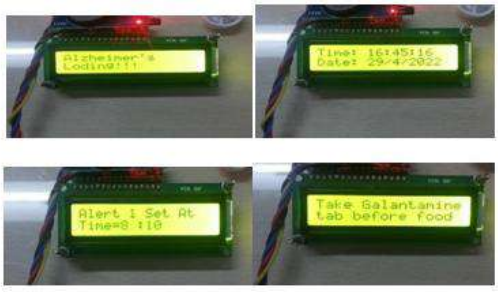

Figure 5: Outcomes

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RESEARCH ARTICLE

Zinc-Induced Impact on Productivity, Zinc use Efficiency and Grain Biofertication of Rice under Zinc Stressed Soil

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ABSTRACT

Zinc (Zn) deficiency is a global concern for human health and causes a decrease in crop production and nutritional characteristics. A field experiments were conducted in a zinc deficient soil belonging to Vertisol (Typic Haplusterts) and Entisol (Typic Ustifluvents) during Rabi 2011-2012. To study response of rice to the integrated use of zinc and organics.The treatments consists of four levels of zinc viz., 0, 2.5, 5.0 and 7.5 mg kg^{-1} and organic sources viz., no organics, FYM @ 12.5 t ha^{-1}, green manure @ 6.5 t ha^{-1}, poultry manure @ 10 t ha^{-1} and vermicompost @ 5 t ha^{-1}. The test crop was rice var.ADT 36. The results revealed that grain and straw yield was significantly enhanced on addition of zinc or organics or both over control in both soils. The rice yield increased with zinc doses and maximum yields was noticed with 5 mg Zn kg^{-1} and declined at 7.5 mg Zn kg^{-1}. While addition of poultry manure recorded the maximum rice yields and was on par with vermicompost. However the highest grain yield (6103, 6344 kg ha^{-1}) and straw yield (8369, 8459 kg ha^{-1}) was recorded with application of 5 mg Zn kg^{-1} and poultry manure @ 10 t ha^{-1} in Vertisol and Entisol respectively. Zinc use efficiency (agronomic efficiency and apparent zinc recovery) was highest at 2.5 mg Zn kg^{-1} and declined with loading of zinc. Zinc use efficiency increased further in the presence of organics and the maximum ZnUE was noticed with 2.5 mg Zn kg^{-1} and poultry manure and was comparable with vermicompost.

Keywords: Zinc, organics, rice, yield, ZnUE.

INTRODUCTION

Staple cereals (wheat, rice, and maize) are the principal source of food in developing countries with low amounts of micronutrients including zinc (Zn), boron (B), and iron (Fe) [Nadeem and Farooq, 2019]. Therefore, the use of only staple food in daily diet is a major cause of widespread micronutrient deficiency in under-develop countries [Eradal et al.,2019].Rice is grown in diverse soil and water regimes, consequently depletion and toxicity of micronutrients is encountered in many parts of India. Zinc deficiency in soil is influenced by many factors which includes pH, concentration of Zn, Fe, Mn and P in soil solution, $CaCO_3$ (Brar and Sekhon, 1976). Integrated use of organics and zinc have been found to more effective in maintaining higher productivity and stability through correction of deficiency of zinc in the course of mineralization on one hand and favorable physical soil condition on other hand. Organic manuring improves rice yield and ZnUE when applied in conjunction with Zn fertilizer. Mirza et al., (2010) reported higher rice yield when organics was applied with $ZnSO_4$ compared to Zn alone. Keeping in view of the importance of zinc nutrition and its use efficiency in rice and use of organics in maintaining soil fertility, field experiments were conducted in two soils deficient in zinc to study the effect of zinc and organics in lowland rice.

MATERIALS AND METHODS

Field experiments were conducted during Rabi 2011-2012 in Vertisol (Typic Haplusterts) and Entisol (Typic Ustifluvents). Before imposition of treatments, the soil used in the experiment had the following properties viz., pH-8.2: 7.5, EC-0.49:0.73 dSm^{-1}, organic carbon-4.65:5.36 g kg^{-1}, CEC-42.1:21.2 c mol(p^+) kg^{-1},$CaCO_3$- 3.38:1.37%, $KMnO_4$-N- 329:296.3kg ha^{-1}, Olsen-P- 31.2:16.0 kg ha^{-1}, NH_4OAc-K- 330.8:299 kg ha^{-1} and DTPA-Zn-0.73:0.59 $mgkg^{-1}$(Vertisol and Entisol). The treatments consisted of four levels of zinc viz., 0,2.5,5.0 and 7.5 mg kg^{-1} applied through $ZnSO_4$ and organics viz., no organics, FYM- 12.5 t ha^{-1}, green manure- 6.5 t ha^{-1}, poultry manure – 10 t ha^{-1} and vermicompost- 5 t ha^{-1}. The design was FRBD with three replications. Twenty seven days old rice seedling var ADT 36 was transplanted in the main field. All the plots received uniform dose of 150 kg N ha^{-1}, 50 kg P_2O_5 ha^{-1} and 50 kg K_2O ha^{-1} applied through urea, SSP and muriate of potash respectively. Grain and straw yield was recorded at harvest and expressed as kg ha^{-1}. The plant samples were analyzed for zinc following standard procedure. Soil was analyzed for DTPA zinc (Lindsay and Norwell, 1978). Zinc use efficiency was calculated following the formula proposed by Fageria (2009).

RESULTS AND DISCUSSION

Rice yield

Analysis of variance(p=0.05) on rice yield showed that application of graded dose of zinc or organics or both significantly enhanced the grain and straw yield over control(Table1). Addition of 5 mg Zn kg^{-1} registered the maximum grain yield (5600, 5910 kg ha^{-1}) and straw yield (7783, 7893 kg ha^{-1}) which was about (15.6, 14.3%) and (20.7, 20.9%) greater than control (no zinc) in Vertisol and Entisol respectively. The rice yield declined at 7.5 mg Zn kg^{-1}. The higher rice yield due to zinc is attributed to its involvement in many metallic enzymes system, regulatory function and auxin production (Hacisalihoglu *et a*l, 2002), enhanced synthesis of carbohydrates and their transport to the site of grain production (Pedda Babu *et al*., 2007). Among organics, addition of poultry manure@ 10 t ha^{-1} reported the highest grain yield (5742, 6039 kg ha^{-1}) and straw yield (7951, 8084 kg ha^{-1}) in Vertisol and Entisol respectively and was comparable with vermicompost. The percent increase due to poultry manure on grain yield (23.1, 22.0) and straw yield (22.2, 21.9) was noticed over control in Vertisol and Entisol respectively. This could be due to supply of nutrients especially macro and micronutrients which induce cell division, expansion of cell wall, meristamatic activity, photosynthetic efficiency, regulation of water into cells, conducive physical environment leading to better aeration, root activity and nutrient absorption resulting in higher rice yield(Singh *et al*., 2001). In the present study, poultry manure contained higher nutrient content compared to other organics which caused higher rice yield. Higher grain yield due to poultry manure was reported by (Sangeeta *et al*., 2013). Interaction

between zinc and organics was significant. The highest grain yield (6103, 6344 kg ha^{-1}) and straw yield (8369, 8439 kg ha^{-1}) was observed on addition of 5 mg Znkg^{-1} and poultry manure @ 10 t ha^{-1}. It caused (18.2, 16.3%) and (25.1, 20.0%) increase in grain and straw yield over control. This is due to the fact that zinc availability was expected to enhance through complexation or chelation thereby prevent fixation in soil (Latha *et al*., 2001).

Zinc use efficiency

Zinc use efficiency (ZnUE) was significantly influenced by addition of zinc and organics (Fig.1). Agronomic efficiency, physiological efficiency, agro physiological efficiency, apparent zinc recovery and zinc utilization efficiency was highest at 2.5 mg Zn kg^{-1} and declined with Zn loading. It was due to inverse relationship observed between utilization and rate of application and also due to progressive decline in grain yield at highest level of zinc applied.(Fageria, 1992) Higher ZnUE at lower level of Zn in rice was reported by Fageria *et al*., (2011). Apparent zinc recovery was very low due to poor distribution from low rates applied and fertilizer reaction with soil to form insoluble products.(Mortvedt,1994). BrijeshYadav *et al.*,(2013) reported higher recovery of zinc at lower level of zinc applied. Addition of organics also improved ZnUE over no organics and the maximum value was noticed with poultry manure and was comparable with vermicompost. Zinc use efficiency increased further when zinc was applied in the presence of organics compared to their absence and the highest ZnUE was noticed with 2.5 mg Zn kg^{-1} + poultry manure compared to other combinations. Zinc enriched organic manures improves the availability of zinc in soils by preventing their fixation and precipitation thereby enhances the use efficiency of applied zinc (Sathynarayana et al., 2002)

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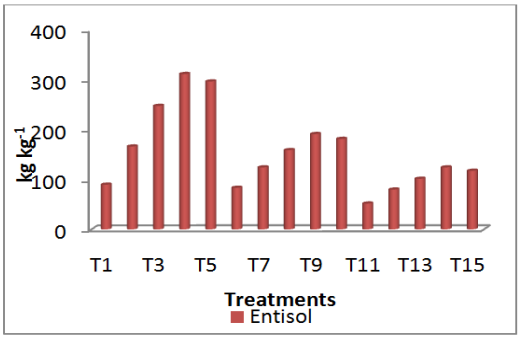
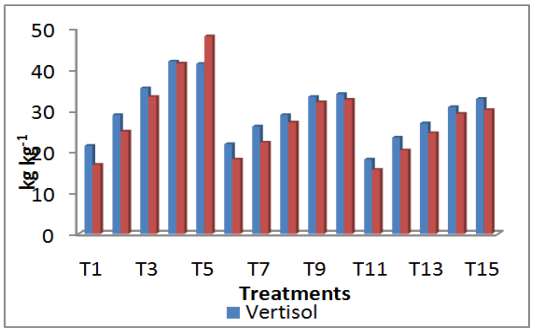
Table. 1. Effect of zinc and organics on grain and straw yield (kg ha^{-1})

Organic sources	Grain yield					Straw yield				
	Zinc levels(mg kg^{-1})									
	0	2.5	5.0	7.5	Mean	0	2.5	5.0	7.5	Mean
	Vertisol									
O_1 - Control	4107	4536	4930	4806	4595	5824	6451	6874	6865	6504
O_2 – FYM @ 12.5 t ha^{-1}	4506	4871	5295	5200	4968	6315	6929	7484	7419	7037
O_3-GM @ 6.25 t ha^{-1}	4855	5270	5596	5502	5306	6753	7385	7940	7881	7490
O_4 –PM @ 10 t ha^{-1}	5149	5678	6103	6058	5747	7299	7813	8369	8324	7951
O_5-VC @ 5 t ha^{-1}	5074	5623	6077	5818	5648	7151	7811	8246	8204	7853
Mean	4738	5196	5600	5477		6668	7277	7783	7739	
		O	Zn	O xZn		O	Zn	O xZn		
SEd		58.05	66.85	116.11		50.48	54.06	120.88		
CD(p=0.05)		116.69	134.37	233.39		101.48	108.66	242.97		
	Entisol									
O_1 - Control	4432	4880	5265	5210	4948	6559	6559	7013	6966	6633
O_2 – FYM @ 12.5 t ha^{-1}	4785	5263	5675	5631	5338	6604	7106	7542	7510	7190
O_3-GM @ 6.25 t ha^{-1}	5198	5670	6020	5955	5711	7044	7543	8020	7962	7642
O_4 –PM @ 10 t ha^{-1}	5527	5990	6344	6297	6039	7474	8000	8459	8403	8084
O_5-VC @ 5 t ha^{-1}	5457	5915	6246	6195	5953	7342	7931	8433	8385	8023
Mean	5080	5544	5910	5808		6891	7428	7893		
		O	Zn	O xZn		O	Zn	O xZn		
SEd		50.40	56.02	123.69		55.03	59.35	128.42		
CD(p=0.05)		101.31	112.61	248.62		110.63	119.31	258.13		



(A)



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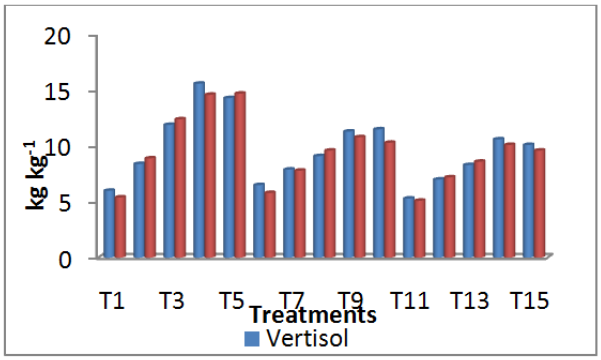

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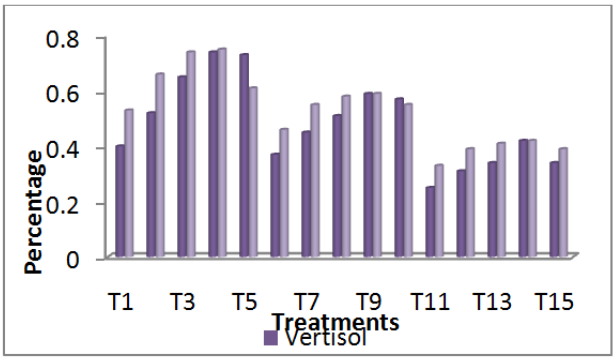

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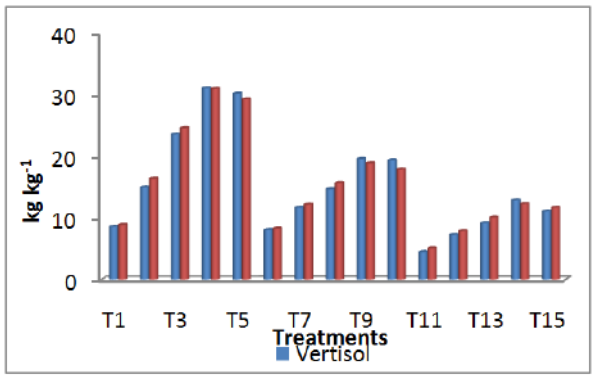

(E)

T_1- No organics + 2.5 Zn(mg kg^{-1}) T_6- No organics + 5.0 Zn(mg kg^{-1}) T_{11}- No organics + 7.5 Zn(mg kg^{-1})

T_2- FYM + 2.5 Zn(mg kg^{-1}) T_7- FYM + 5.0 Zn(mg kg^{-1}) T_{12}- FYM + 7.5 Zn(mg kg^{-1})

T_3- GM + 2.5 Zn(mg kg^{-1}) T_8- GM + 5.0 Zn(mg kg^{-1}) T1_3- GM + 7.5 Zn(mg kg^{-1})

T_4- PM + 2.5 Zn(mg kg^{-1}) T_9- PM + 5.0 Zn(mg kg^{-1}) T_{14}- PM + 7.5 Zn(mg kg^{-1})

T_5- VC + 2.5 Zn(mg kg^{-1}) T_{10}- VC + 5.0 Zn(mg kg^{-1}) T_{15}- VC + 7.5 Zn(mg kg^{-1}

Fig.1.Effect of zinc and organics on zinc use efficiency. A) Agronomic efficiency, B) physiological efficiency, C) Agro physiological efficiency, D) Apparent zinc recovery, E) Zinc utilization efficiency.

REVIEW ARTICLE

Therapeutic Potential of Polyphenolic Nano-Based Strategies: A Review

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ABSTRACT

Polyphenols are the class of natural compounds present in all vascular plants possessing scavenging properties towards radical oxygen species. Phenolic compounds can be divided into two main groups are flavonoids and non-flavonoids. The beneficial health effects of polyphenols are related to several remarkable biological properties, such as antioxidant, anti-inflammatory, cardioprotective, neuroprotective activities etc. Unfortunately, Phenolic compounds having lack in long-term stability, very sensitive to light, low water solubility and poor bioavailability. To overcome the drawbacks, the use of nano-based strategies appears to be a promising method to potentiate the therapeutic action. The objective of this review is to present therapeutic application of nano-based strategies for Polyphenolic compounds.

Keywords: Polyphenol, Flavonoid, Non Flavonoid, Stability, Solubility, Bioavailability, Phenolic Compounds

INTRODUCTION

Polyphenols are widely studied natural bioactive secondary plant metabolites due to their potential positive effects on human metabolic processes. "Phenolic" is a substance that characterized by the presence of one or several aromatic rings bearing one or more hydroxyl substituents. These active principles play an important role in growth, reproduction, resistance to pathogens, predators and diseases. Fruits, vegetables, legumes, cereals, cocoa, coffee, tea, and wine are well-known food items rich in polyphenols [1]. Polyphenols have attracted the interest of many researchers due to thepotential health benefits to humans. The beneficial therapeutic effects of polyphenols are related to severalbiological properties, including antioxidant, anti-inflammatory[2], cardioprotective [3], and neuroprotective activities [4-5]. Furthermore, polyphenols are able to inhibit bacterial [6], fungal [7], or viral infections[8].Also inhibit the development of tumors[9, 10], and interact with a broad number of proteins, such asenzymes, tissue proteins, and membrane receptors [11, 12]. modulating their activity in a specific way. Polyphenols have low bioavailability due to many intrinsic and extrinsic factors, including their chemical structure and molecular weight, poor water solubility, low stability in the gastrointestinal environment, extensive phase II metabolism and rapid elimination[13, 14]. To avoid these limitation, nano based delivery systems able to maintain the structural integrity of the bioactive molecules have been developed [15-17].

Classification of Polyphenols

Based on the number of phenol rings they contain and the structural components that hold these rings together, polyphenols are categorised. Phenolic acids, flavonoids, stilbenes, and lignans are the four broad categories into which they are divided [3].

Therapeutic Potential of Polyphenols

Cardiovascular Health

Due to the ability of polyphenols (flavonoids, anthocyanidins) to inhibit LDL oxidation, platelet aggregation and adhesion, inflammatory response of the vascular tissues, and inducing endothelium-dependent vasodilation, risk of developing cardiovascular disease decreases [18]. Cassidy et al. studied participants from the Nurses' Health and Health Professionals Follow-up Studies and found that participants in the highest anthocyanin intake (from blueberries and strawberries) had an 8% reduction in risk of hypertension [19]. Flavonoids (from berries, fruits and vegetables) have been shown to reduce risk factors of cardiovascular disease such as blood pressure [19, 20], arterial stiffness [20], total LDL cholesterol, and plasma adhesion molecules [21, 22]. Oxidation of LDL (oxLDL) particles in the arterial wall is considered a key event in atherosclerosis [23]. Basu et al. challenged 36 women with metabolic syndrome with light Cranberry juice for 8weeks. They reported that oxLDL was decreased from baseline compared to placebo [24]. Flavonoids have anti-platelet activation and anti-platelet aggregation abilities that may occur through mechanisms such as increasing prostacyclin, inhibiting phosphodiesterases that degrade cAMP [25-27].

Cancer

Numerous scientific studies from the literature have proven the cancer-preventive action of tea consumption [28]. Therapeutic activities of Epigallocatechin gallate is attributed to a plethora of mechanisms, including modulation of enzymes responsible for metabolism of carcinogens and cell signalling pathways, stimulation of apoptosis arrest of cell cycle, and inhibition of stimulation of transcription factors leading to suppression of development and progression of cancer [29]. Treatment of human lung cancer A549 cells with pomegranate fruit extract (contains punicalagin and ellagitannins), at concentrations of 50–150ppm, led to cytotoxic effects with arrest of cell cycle in the G0–G1 phase, reduced expression of cyclins and cyclin-dependent kinases, and enhanced cell cycle regulatory proteins. It was found to suppress PI3K, MAPK, Akt, and NF- κB phosphorylation [30]. Several studies have reported inhibition of tumor proliferation by silybinin in a variety of cancers, ranging from prostrate, ovarian, skin, breast, and bladder to lung cancer [31]. Exposure of NSCLC cells to curcumin reduce the invasion and proliferation of these cells, and surged arrest of the G0/G1 phase cell cycle through blockade of metastasis-associated protein 1 regulated inhibition of the Wnt/β-catenin pathway [32]. Different phenolic compounds present in mushroom such as

phenolic acids, flavonoids etc which exhibit anticancer potential. Methanolic extract of *Agaricusbisporus* inhibits human colon cancer cell line (Colo-205) and breast adenocarcinoma cell line (MCF7) [33]. Methanolic extract of *Agaricuslanipes* inhibits human lung adenocarcinoma (A549) [34].

Inflammation and Immune Function

Obesity is defined as a condition of systemic inflammatory response associated with an altered immunity [35]. In vitro, flavonoids were able to inhibit transcription factors as well as differentiation of pre adipocytes into mature adipocytes [36]. A wealth of information is available on the therapeutic effectiveness of polyphenols in the course of allergic pathologies. With particular reference to polyphenols, some evidence has indicated that curcumin (a flavonoid) is able to exert aldulticidal effects on *Schistosoma (S.) mansoni* and limit the extension of hepatic granuloma in animals infested with *S. Mansoni* [37]. Quercetin affects immunity and inflammation by acting mainly on leukocytes and targeting many intracellular signaling kinases and phosphatases, enzymes, and membrane proteins often crucial for a cellular specific function [38]. Quercetin (a flavonoid) is able to abrogate Ni-mediated increase in oxygen radicals and decrease in histone acetylation [39]. The inhibition of ROS production, a possible antioxidant mechanism for flavonoids can proceed by direct binding to the enzyme or by ROS scavenging. Flavones and flavonols display a general affinity to ATP-binding proteins as a consequence of their formal structural analogy with ATP [40].

Gastrointestinal and Liver Disease

Studies showed that pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β and reactive oxygen species and myeloperoxidase (MPO) production were reduced by green tea polyphenol (epigallocatechin-3-gallate) administration [41]. Bilberry and bilberry extract (contains anthocyanins) reduced colonic inflammation scores of DSS induced colitis with decreased production of IFN-γ and IL-6 from mesenteric lymph nodes [42]. In vitro studies with liver slice culture have shown that curcumin decreased lipid peroxidation, reduced the release of LDH, and attenuated the antioxidant enzymes SOD, CAT and GSH-Px[43]. Studies have shown that curcumin mitigated oxidative stress and prevented liver cell damage in experimental animals [44, 45]. Rafatullah et al. have shown that the oral administration of 500mg/kg of the ethanolic extract of turmeric produced significant anti-ulcerogenic activity in rats subjected to indomethacin administration [46]. y Long and coworkers have also shown that treatment with curcumin prevented reserpine-induced tissue damage and cell death by decreasing inflammatory response and modulating the expression of VIP and gastrin [47]. Studies have shown that turmeric prevents chemical-induced gastric carcinogenesis in experimental animals [48].

Skin Disease

Polyphenols are increasingly being unravelled for their nutritional, therapeutic, and cosmetic applications. Tannic acid, a type of polyphenol from different plants including pomegranate (*P. granatum*) and *Hibiscus cannabis* flowers were found to be effective inhibitors ofrecombinant glutathione transferase of Egyptian cattle tick, *Rhipicepalus (Boophilus) annulatus* [49]. Catechins (polyphenol) could be used as novel topical skin remedies against HSV-1[50]. Polyphenols help maintain dermal elasticity and natural vibrancy of skin, thus improving the overall skin contour. Green tea polyphenols inhibit UVB-induced immune suppression and inhibit dermal cancer [51]. Polyphenols have strong antioxidant and free radical scavenging and antimicrobial properties. So, polyphenols used in cosmetics, lotions, skin and hair creams, and topical preparations. The CGA (chlorogenic acid) extracted from coffee, is proposed as an able preventive agent against UV-mediated premature aging of skin [52]. Polyphenols have health benefits in inflammatory associated diseases, and growing evidence reports their action on dendritic cell phenotype and function, thus indicating the greater potential of the polyphenols in DC-based immunotherapeutic approaches [53, 54].

Bioavailability of Polyphenols

Bioavailability means that the fraction of the dose that reaches the systemic circulation according to the route of administration. The bioavailability of polyphenol is rather low after oral administration, due to different factors such as, poor solubility, rapid metabolism, membrane permeability, large size and incompatibility [55]. To overcome these

drawbacks, nano-based drug delivery systems and special formulations have been developed. Polyphenols are prone to degradation in the gastrointestinal tract. Polyphenol nanoparticle can prevent degradation in the gastrointestinal tract.

RESULTS AND DISCUSSION

Curcumin

Curcumin is a polyphenol which is found in the rhizome of Curcuma longa, i.e., turmeric [56]. Curcumin is found to have antioxidant activity, anti-inflammatory property, anti-arthritic, osteoarthritic effect,anticancer and antibacterial effect [57-62]. Curcumin is said to attenuate various metabolic syndromes such as obesity, hypertension, insulin resistance, and hyperglycemia[63-66].

Epigallocatechin-3-gallate (EGCG)

Epigallocatechin-3-gallate is a water-soluble polyphenol found in leaves of *Camellia sinensis*. It is a strong natural antioxidant, made up of epigallocatechin and gallic acid[75]. EGCG have positive effect in the treatment of chronic lymphocytic leukemia, multiple sclerosis [76], cardiovascular diseases, type 2 diabetes mellitus, osteoporosis, liver fibrosis, cirrhosis, and Alzheimer's disease [77].

Apigenin

Apigenin is a bioflavonoid compound that is mostly found in herbs and also in plants. Apigenin can exert antianxiety property [84], neuroprotective effects [85], and anti-inflammatory property [86], antidiabetic activity [87], anti carcinogenic activity [88].

Eugenol (EUG)

Eugenol is a bioactive phenolic compound habitually occurs in herbal plants clove, cinnamon, tulsi, and pepper etc. A number of therapeutic effects like antiviral, antifungal, antioxidant, anti-inflammatory, and anticancer have been reported.

Quercetin (QC)

Quercetin (3,3′,4′,5,7-pentahydroxy flavone) is the most potent polyphenolic flavanoid having biological, pharmacological, and medical benefits [103]. Quercetin is promoted for prevention and treatment of cancer and this principle is utilized as a nutritional supplement and as a phytochemical remedy for a variety of diseases like diabetes, obesity, circulatory dysfunction, and inflammation and mood disorders [104].

Resveratrol

Resveratrol is a naturally occurring compound, belongs to stilbenes class of polyphenol. Resveratrol produced by a wide variety of plants in response to injury, UV Irradiation, ozone exposure and fungal attack [112]. A number of therapeutic effects like antiplatelet activity, vasodilating property [113], anticancer [114], anti-inflammatory [115], cardioprotective[116], neuroprotective effects [117] have been reported. Polyphenols are valuable compounds present in plants, fruits, legumes, chocolate, tea, wine and marine organisms possessing scavenging properties. They show a unique combination of chemical, biological and physiological activities. Unfortunately, polyphenols have short-term stability, are very sensitive to light, and often present a poor aquous solubility and bioavailability. To overcome these limitations and enhance polyphenols therapeutic applications, nano-based strategiess have been developed. The various reported research revealed that physicochemical nano-based strategy provided a significant protection against drastic conditions such as oxidation and thermal degradation, thereby contributing to increase the shelf-life of the active ingredients. Furthermore, nano-based strategy are also able to control the release, change the physical properties of the initial material, and improve the bioavailability of the polyphenols.

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Table 1: Nanodelivery Systems of Curcumin and their therapeutic use

Type of Delivery Systems	Therapeutic use
Curcumin-loaded chitosan nanoparticle [67].	Anticancer activity
PLGA-poloxamerblend nanoparticles [68].	Induce cell cycle arrest in mesothelioma cells
Transferrin mediated solid lipid nanoparticles [69].	Anticancer activity
Human Serum Albumin nanoparticles [70].	Anti tumor activity
β-cyclodextrin nanoparticle [71].	Enhance Skin Permeability
Micelle [72].	Anti tumor activity
Nanocomposite particles [73].	Enhanced target specificity and potency
Liposome [74].	Anti-inflammatory Activities

Table 2:Nanodelivery Systems of Epigallocatechin-3-gallate and their therapeutic use

Type of Delivery Systems	Therapeutic use
EGCG-loadedCS–CPP nanoparticles [78].	Enhance antioxidant activity.
Epigallocatechin gallate-loaded polysaccharide nanoparticles [79].	Prostate Cancer.
Nanolipidic EGCG particles [80].	Alzheimer's disease.
PLGA nanoparticles [81].	Antioxidant activity.
EGCG anionic nano Liposomes [82].	Act as antioxidant in stress and oxidative damage.
Molecularly imprinted polymeric coated electrode [83].	Anti-aging and cancer.

Table 3: Nanodelivery Systems of Apigenin and their therapeutic use

Type of Delivery Systems	Therapeutic use
Nanocrystals [89]	Antioxidant activity.
Carbon nanotubes [90]	Enhanced bioavailability.
Nano-range Distearoylphosphatidylcholine liposomes [91]	Antibacterial activity.
PEGylated PLGA nanoparticles [92]	Anti-inflammation and anti-fibrosis.
Ethosomes[93]	Treatment of UVB induced skin inflammation.
Bovine serum albumin nanoparticles [94]	Antioxidant and anti-inflammatory in lung injury.
PLGA nanoparticles [95]	Skin melanoma.

Table 4: Nanodelivery Systems of Eugenol and their therapeutic use

Type of Delivery Systems	Therapeutic use
Chitosan nanoparticles [96]	Antioxidant and antibacterial properties.
Nanoemulsion[97]	Anti cancer effects in colon and liver cancer.
Nanocapsule[98]	Periodontal infections.
Chitosan-coated PCL nanoparticles [99]	Cerebral ischemia.
PLGA nanoparticles [100]	Antimicrobial effect.
Solid lipid nanoparticles [101]	Antifungal activity.
Nanostructured systems [102]	Anti-inflammatory and antioxidant effect.

Table 5: Nanodelivery Systems of Quercetin and their therapeutic use

Type of Delivery Systems	Therapeutic use
PLGA nanoparticles [105]	Antidiabetic effect.
Nanocapsule[106]	Arsenic-induced hepatic and cerebral oxidative damage.
Biocompatible nanoparticles [107]	Anticancer.
Nanocapsule[108]	Anti inflammation and anti ulcer.
Liposomes [109]	Anxiety and cognitive effects.
β-cyclodextrines[110]	Antioxidant.
Nanoliposomes[111]	Hepatocellular carcinoma.

Table 6: Nanodelivery Systems of Resveratrol and their therapeutic use

Type of Delivery Systems	Therapeutic use
CS-TPP nanoparticles [118]	Antiproliferative activity.
Solid lipid nanoparticles [119]	Greater intracellular Delivery, in skin disease
Niosomes[119]	Antioxidant in skin disease
Biodegradable nanoparticles [120]	Anticancer.

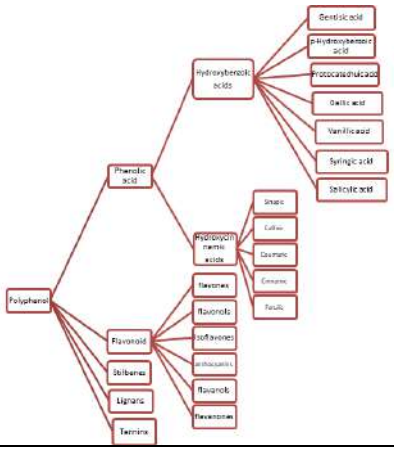

Figure-1: Main Classes of Polyphenols

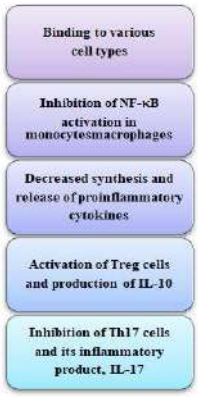

Figure-2: Anti inflammatory activities performed by polyphenols.

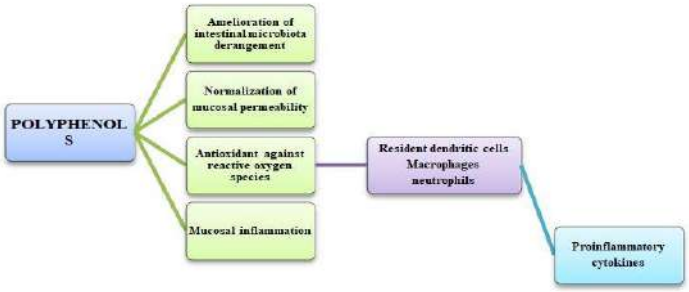

Figure - 3: Immunological features of colitis and its treatments

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RESEARCH ARTICLE

Localization of Number Plate using Deep Learning Models

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ABSTRACT

Automatic Number Plate recognition system has evolved drastically over past decade. It involves localization of the plate followed by recognition of characters in the plate. The key process in the recognition system is the number plate localization from the input images. We proposed a deep learning-based model to localize the number plate. The proposed system uses U-Net architecture which enables the precise localization of the plates with the help of contextual information from the input images. The Region of Interest(ROI) created by the model is further validated with the help of Convolution Neural Network (CNN) to make the system more reliable for true positive outcomes. The CNN which extracts the representation vectors from the images and classify the segmented ROI either a plate or not a plate. For the proposed system, a robust real time vehicle dataset with different background in unconstrained environment was developed. The proposed system was evaluated using different measures such as IOU (Intersection Over Union), Precision, Recall and Accuracy measures.

Keywords: CNN, Deep learning, Number Plate Localization, Segmentation and U-Net.

INTRODUCTION

In recent years Automatic Number Plate Recognition (ANPR) Systems have evolved to adapt to various applications. It finds applications in various fields such as traffic management, automated vehicle entry system, toll collection and surveillance. There were several methods proposed in the literature to solve this problem in recent years. All solution

primarily consists of three major subsystems: Plate localization system, character segmentation system and Optical Character Recognition system. The latter two subsystems, though is an important part of ANPR, does not pose any serious impact to the overall performance of the system. The plate localization system is relatively a difficult task and its performance can objectively decide whether the system will produce an output or not. Even though they are many systems developed for ANPR two main factors that contribute to the challenges of ANPR such as plate variations and the environment variations. The plate variations are due to the fact such as location, orientation and size of the license plate in a given image. There are different variety of license plate designs across different jurisdictions in terms of color and style of the license plates. The ANPR system is considered to be computationally intensive, error prone and to have long processing time. Thus most of the proposed systems, localises the number plate better with trivial images than real world test-cases. There may also pitfall in the accuracy of the ANPR systems since the input image in the real world don't get proper illumination and the images taken in dark are predominantly masked with salt and pepper noise. In the due process of enhancing the image quality, the image sometimes loses valuable information. This makes it a really difficult to localise the plate from the images that has comparatively minimum contrast between the foreground and background pixel values. Though Adaptive thresholding has proven to perform better in most of the test cases, it failed in images with multiple foreground mimicking blobs. Thus, a suitable solution to handle the unconstrained environment such as plate variation and environment variation needs to be addressed with the help of features being learnt from the images. In the literature, many work have been carried out for ANPR systems using different methods, a comprehensive review of state of art techniques have been discussed [1]. An automatic vehicle license plate recognition method for Western Australia license plates was proposed in [2] where the plate extraction method uses Roberts edge detection method followed by morphological operators. Harris corner detection method have been used in [3] which manages the corners even in diverse brightening conditions. The rectangular license plate region is obtained using edge-based image processing methods with the help of skew correction have been discussed [4].

Potential plate region have been localized using Bottom-Hat filter followed by open and close operations with skew correction have been proposed in [5]. A modified Graph-cut algorithm with addition to feature extraction method performs accurate foreground extraction based on the geometric information have been proposed in [6]. Genetic algorithm-based method was proposed in [7] to localize the number plates with image processing along with color feature extraction. Sobel edge detection algorithm was used over the gray scale image followed by a morphological close operation [8], the edges were then reconstructed using connected component method. Harris corner detection over statistically most probable region was performed in [9], the plate was then extracted using sobel edge detection technique. In recent years many deep learning based algorithms were proposed for object detection. All the proposed deep learning models for object detection has a region proposal algorithm and a proposal validator algorithm. RCNN uses selective search algorithm [10] to obtain 2000 proposals from the image. These proposals are validated by a CNN. A modified version of R-CNN was used [11] and it does not uses a CNN to validate the proposals, rather it obtains the feature maps of the image and uses the feature maps to obtain the ROI. Both R-CNN and Fast R-CNN uses selective search algorithm to obtain the region proposals, but in Faster R-CNN [12] a separate learning network is used to predict the proposals and these predicted feature map regions are passed to a ROI layer to reshape them to standard size. U-Net[13] uses the feature vectors from the image to construct a segment mask for the ROI. From the literature it was found that static algorithms may not provide good accuracy for the images obtained from unconstrained environment but deep learning-based algorithm are found to be performing better since the model learn features from the given image. Thus, we proposed a U-Net based deep learning architecture to localise the plate from the image. The model follows a two-stage pipe lined architecture, where the binarised image is provided to the U-Net model to generate a mask that will be used to localise the plate. The second stage is a CNN validator that validates whether the segmented area is actually a plate or not.

MATERIALS AND METHODS

In this research work, we proposed deep learning model to segment and localise the plate from the input images. The input vehicle images are processed and the U-Net model is used to generate a mask for the number plate region. The output of the plates are further post-processing using morphological operations. The number region are localized from the masked region and it can further validated as a plate or not using CNN. The localised images are further validated using CNN model to reduce false positive outcomes of the proposed system. The proposed system is depicted in Fig. 1. The input images have been obtained from unconstrained environment with different illumination and background. For an unconstrained environment, a static algorithm like edge detection and thresholding will not produce good segmentation results for all images. We propose a two stage pipeline based on deep learning technique to segment and localise the number plate which is further validated using a CNN based classifier. The model learns the features from the images leading to good performance. The algorithm for the proposed system was also shown below.

Algorithm 1:Number Plate Localization
Require: input image $x \in R^m \times R^n$
$x \leftarrow$ grayscale(x)
$x \leftarrow$ resize(x,(256, 256)) $x : R^m \times R^n \rightarrow R^{256} \times R^{256}$
mask $\leftarrow$ fU−Net(x, θU−Net model)
processed mask $\leftarrow$ morph(mask)
processed mask $\leftarrow$ resize(mask,(m, n))
edges $\leftarrow$ Canny(processed mask)
contours $\leftarrow$ find contours(edges)
ROI is the localised area from the contour.
if f_{CNN} (ROI, $\theta_{CNNModel}$) = 1 **then**
    return ROI
else
    Plate not found
end if

Plate Segmentation
In segmentation we aim to distinguish the region of interest from rest of the background information for easier localisation. The plate segmentation is carried using U-Net model. The U-Net architecture which is called fully convolutional network can be considered to be the best CNN based architecture to segment regions of the image as different classes. The model predicts a single channel segmentation map for the input image. In normal classification problem, using traditional CNN the feature map are vectorized and vectors are used for classification. However, in image segmentation, a mask has to be reconstructed from the feature map vectors. This is possible only with the help of symmetry architecture like U-net which consists of contracting path and expanding path.

The contracting path is composed of several blocks which contains convolution layer followed by max pooling layer. The feature map size increases exponentially from one block to another block which helps the architecture to learn complex structures effectively. During contraction phase high dimensional images are decomposed to low dimensional feature vectors. The bottleneck layer between these two phases of the network, constricts the amount of information flow so the contraction layers learn better representations. The expansion architecture is symmetric to the contraction architecture with same number of blocks. These feature vectors from the contraction phase along with information from corresponding skip connections are used to reconstruct high resolution segment maps in the expansion path. This preserves the structural integrity of the image and reduces the distortion. The pooling operation is replaced by upsampling operation in the expansion network. Furthermore, during expansion there are relatively more number of convolution operations performed to predict an accurate segmentation map. The final

activation layer in the neural network is softmax which is applied for a single channel output. Using the output from the U-Net a binary map has been constructed that represents the plate region in the image. Let $a(x)$ be the output of the U-Net architecture. We define binary map p(x) as, Let a(x) be the output of the U-Net architecture. We define binary map p(x) as,

$$p(x) = \begin{cases} 1, if a(x) > \delta \\ 0, \ otherwise \end{cases} \quad (1)$$

The pixel "x" belongs to plate region, if a(x) is greater than a threshold δ. During training we aim to minimise the deviation of the output channel from the ground truth. This deviation is quantified by the cross entropy defined in Eqn. 2

$$E = \sum_{x \in \Omega} w(x) \log Pplate(x) \quad (2)$$

where w(x) is the weight map to give each pixel specific importance. Since in this application, both the classes are given equal importance, we keep w(x) = 1 ∀x. The error calculated is back propagated to calculate the gradients for the weights in the hidden layers. The three-channel input image of our proposed system is converted to a single channel gray scale image and resized to 256 × 256. After resizing, the image is binarized and passed to the U-Net architecture. The contracting path of the architecture captures the context of the input image. The coarse information obtained from the contracting path are fed into the expanding path by skip connections, enables precise localization at expanding path. In this proposed work, the U-Net architecture consists of 4 blocks in each contracting and expanding path. Each block consists of 2 convolutional layer followed by max pooling layers. The activation function used at the convolutional layers is ReLu. The bottle neck layer, which mediates between the contraction and expanding layer, consists of 2 convolutional layer followed by a dropout layer. Each expanding block consists of convolutional layer followed by skip connection with corresponding contracting layer followed by convolutional layer. The output of the UNet Model is a 256 × 256 binary mask images and it have been depicted in the fig 2.

Post Processing of Mask and Plate Localisation

The mask obtained from the U-Net may consists of noise to a certain degree. Thus, a morphological close operation is performed to eliminate such region from the mask. The mask was resized back to the original size of the image in order to get the exact location of ROI in the image. The ROI is the rectangular region tha tmaps to the location of the number plate in the image. A Canny edge detection algorithm was used to delineate the edges of the ROI from the mask and bounding box for that region was obtained. The location of the bounding box was used to localise the plate from the original image.

Plate Validation

The segmented plate after localisation must be validated further. In general, most of the segmentation model discussed in literature might fail in unconstrained environment, as such models are not generalised to all possible input distribution. To overcome this, numerous work has been performed in context to deep learning to decrease the generalisation error to make the deep learning model more robust to varying conditions. In our work, we aim to validate the output from the previous stage to reduce the number of false positive outcome. This is important because in any number plate recognition system, plate localisation is the first stage and if there is a error is in the first stage it will have a cascading effect in the later part of the system. Furthermore, our experiments show that the segmentation model is fooled by plate mimicking areas in the image and outputs the segment map accordingly. This is, however, correctly classified by the CNN classifier. Since input images were obtained from unconstrained environment, the proposed U-Net may produce masks at incorrect regions. Hence, we used a CNN based model to validate the masks obtained from the previous stage. Though it is possible to apply the CNN model for every possible 150 * 300 window in the image and avoid using U-Net, but it would be computationally costly algorithm. Thus we employ this model in the later stage of the system to the masks obtained from the U-Net. The proposed

CNN is composed of 2 convolutional layers and 2 max-polling layers placed alternatively, followed by a fully connected layers and an output layer. The architecture of the CNN is shown in Figure. 2. The number plates can be recognised based on the Height: Width ratio and most of the countries follows 1:2 ratio size. So, our proposed CNN model was trained over segmented plate images of size 150 × 300. The CNN learns from vectorised features obtained from the plate image to classify whether the given mask is a plate or not. Thus, the plate localized by the U-Net and canny edge detector is further verified by the CNN model, Whether the obtained mask is a plate or not. This reduces the number of false positive outcomes.

EXPERIMENT

Our proposed system consists of two stages - segmentation of plate and plate validation. We have optimised the learning algorithm which is responsible for each stage and analyse the performance individually and as an end-to-end system. The performance of the U-Net is quantified by IOU and the performance of the validation classifier is quantified by precision, recall, F1 score and accuracy. Furthermore, we measure the overall performance of the system using Success rate.

Dataset

About 1080 vehicle images were collected from various sources with different background and contrast. About 80% of the images were used for training and 20% of the images were used for testing. The ground truth mask of the input images were created with the help of experts. To create the ground truth for each image, the top-left corner and bottom-right corner were identified by the experts and the corresponding mask images (ground truth) of the number plates were generated. In order to analyse the performance of system, we have used different datasets were used to train the U-Net and CNN separately. The U-Net was trained over input images obtained from different background and contrast, either taken from front or rear end of the vehicle. CNN is trained over numerous number plate images to classify whether the proposal made by the CNN has plate or not. Since the number of images for the training purpose were less, a few data augmentation techniques were employed to up-scale the dataset. For about 864 training images, different argumentation techniques such as horizontal flip, rotation by 10◦ and Gaussian noise were used which helps in scaling the dataset to 3456 images. Same augmentation techniques were used to obtain the corresponding masks. For the testing and analysis of the model 130 images were used. The kaggle dataset for Indian number plates shown in Figure.4 were used to train and test the CNN model. The dataset comprises of 1181 number plate images. The dataset was split into training and testing set in 80:20 ratio.

Image segmentation using U-Net

The proposed system uses U-Net architecture to create a masks of the number plate present in the vehicle images. The model was trained for 50 epochs with 6000 batches per epoch and 16 images per batch. The performance of the model was tested with a test data containing 130 images that were never seen by the model during the training phase. In order to analyse the performance of the model IOU (Intersection Over Union) metric was used.
In order to analyse the performance of the model IOU (Intersection Over Union) metric was used.

$$\text{IOU} = \frac{\text{A}_1 \cap \text{A}_2}{\text{A}_1 \cup \text{A}_2} = \frac{\text{Area of Intersection}}{\text{Area of union}} \quad (1)$$

For each of the 130 test images the mask was predicted using the model. The ground truth mask and predicted mask were compared pixel wise to calculate the area of intersection and area of union.

$$A_1 \cap A_2 = \sum_{i=1}^{height} \sum_{j=1}^{width} mask[i][j] \wedge \text{prediction}[i][j] \quad (2)$$

The IOU for each image calculated and the average value was found. The Average IOU value was found to be 80:1%.

It is important to note that IOU is an absolute metric and takes into consideration only the area of overlap. In practice it is observed that, the mask produced is often an extension of the region of Interest ,i.e., the produced mask covers not only the segment where the plate is present, but also the surrounding area around the plate. This accounts to the rough edges of the proposed ROI by the U-Net architecture. Thus low average IOU value merely does not imply the inability of the model to segment the number plate. From Figure. 6, it has been noticed that the produced mask overlaps the ground truth mask. The background plays an important role in determining the ability of system to segment the plate. Inidle case the background can be distinctively identified by either color or texture. Practically, it is often observed that the contrast between the background and foreground object is low. Thus traditional edge detection algorithms, which monitors the gradient change, do not perform well in such situations. The proposed deep learning based strategy obtains pixel information all over the image using convolution process and determines the plate area from it. This makes the system to be more adaptive to different background and amount of illumination.

Post-processing of mask

The mask predicted by the U-Net model may contain some small patches that is too small to containa number plate. Therefore, we apply morphological operation - two iterations of erosion followed by oneiteration of dilation withe kernel size of 5 * 5. Then Canny edge algorithm was used to compute the contours of the patch. We project these contours coordinates to the original image to obtain the number plate from the image.

CNN: Validation of the proposal

The CNN was trained over 945 Indian number plate images. The model was trained for 50 epochs with 8000 batches per epochs, and 32 images per batch. The training was complete with training accuracy of 98:42%. The model was evaluated with 236 plate images that were not used in the training phase. The confusion matrix for the testing phase is shown in table 1. In few cases as in Figure. 5 we observe that the U-Net predicts only partial area of the actual plate tobe the ROI. When such regions are given as input to next stage of the ANPR model, incorrect recognition of the number plate happens. To mitigate such false positive outcomes it is necessary to have an image classifier to validate the objectness of the proposal made by the U-Net. The partial plate predicted by the U-Netin the example shown in Figure. 5 is discarded by the CNN classifier. The inability of the U-Net to identify the exact ROI is due to the fact that plate camouflages with the rest of the vehicle.

Overall performance of the system

The two-stage model was tested with 130 vehicle images, that were used to validate the performance of the U-Net model. The overall performance of the system was quantified using success rate. The success rate of the system is defined as the ratio of number of images that were successfully segmented to the total number of images.

$$\text{Success rate} = \frac{\#\text{ Successful Segmentation}}{\#\text{ Images}} \times 100 \quad (3)$$

For 126 images the U-Net segmentation model was able to segment the plate. The CNN architecture classified 121 out these 126 proposals to be number plates. Out of these 121 predictions 2 were found to be false positive. Therefore, the proposed model was able to localise number plates from 119 images with the success rate of 91.54. The effective success rate of the system is 91.54\%. From Fig. 5, we notice that the U-Net model has segmented plate mimicking regions in few images, however, such false positive outcomes are avoided with the aid of the CNN validations. Since the datasets, used to analyse the performance of methodologies published previously, were not available publicly, a comparative study was not done. Using Otsu's thresholding method and the plate features [5] had a success rate of 54\%. Plate extraction using Roberts edge detection method followed by morphological operation [2] had a success rate of 97\%. Though success rate is high, the paper clearly mentions that, images with poor illumination were not taken into consideration during performance analysis of the system. Harris corner detection method [3] had a success rate of 90\% when tested over 30 challenging images that were taken in different lighting conditions.

CONCLUSION

ANPR is one of the most crucial systems that is used commercially for traffic monitoring. Automated Number Plate Recognition Systems can be briefly characterized by 2 sub-systems, plate segmentation and plate recognition. The algorithm to recognise the contents of the plate image is invariant and mostly consists of an OCR. The plate segmentation on the other hand, is crucial and decides whether the system will produce a meaningful output or not. Thus to achieve good result for ANPR, a reliable segmentation algorithm is necessary. There were many methods proposed to improve the performance of the system in recent years. We propose a deep learning based solution to address this problem in this paper. By using deep learning methods such as U-Net and CNN, we learn the features from the image and try to optimise the solution of localisation. By adapting to a two stage pipeline architecture, it is ensured that the number of false positive outcomes is less. The performance of the system was studied in the presence and absence of a CNN. It was observed that the precision was higher when CNN was used to validate the proposals made by the U-Net model, indicating that the number of false positive outcomes is less.

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Table 1. Confusion Matrix

Confusion Matrix	Plate	Not plate
Predicted as Plate	149	1
Predicted not as plate	20	131

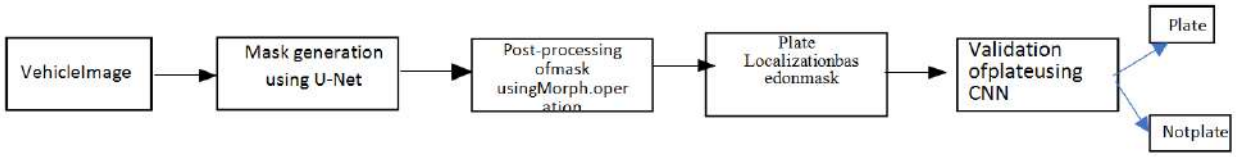

Figure1.Proposed System

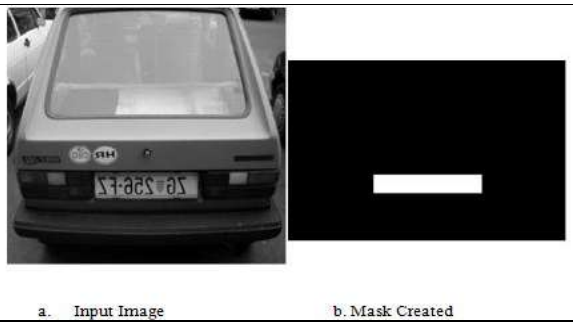

Figure 2. Sample input with Ground truth created

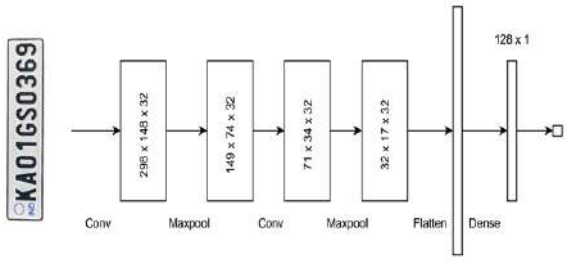

Figure 3. Architecture of CNN

Figure 4. Dataset for CNN

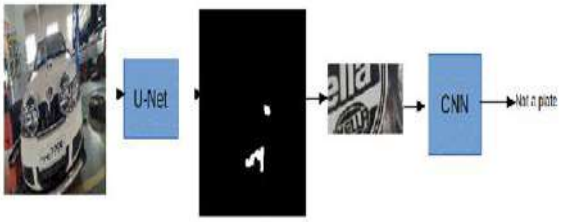

Figure 5. CNN eliminating false positive outcomes

Figure 6. Ground truth(left) and Predicted mask (right)

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RESEARCH ARTICLE

Personality Traits, Social Media usage and Attitude towards Technology in Indian Millennials and Gen Z

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ABSTRACT

The present study aims to study the relationship between the Big five personality traits: Extraversion, Agreeableness, Conscientiousness, Neuroticism, Openness, with social media usage (SMU), and attitude towards technology in young adults which were further divided into Gen Z and Millennial group. The sample consisted of 392 Indian young adults, of whom there was 200 Gen Z population with a mean age of 20 years and 192 millennials with a mean age of 30 years. The sample was administered the MTUAS Scale, BFI-44 Scale, and information about demographic details. The obtained data were analyzed using Pearson's Correlation. The findings show that the mean for SMU is the highest in the Millennials compared to all the other groups. Interestingly, the female population has a higher mean on SMU than the males. The findings indicate that the personality factor of conscientiousness, has a significantly negative relationship with a positive attitude toward technology and anxiety without technology in both the millennials and gen z. On the other hand, openness shows a significant relationship with a positive attitude towards technology and SMU in both the groups. Neuroticism is observed to have a significant relationship with negative attitude towards technology in Gen Z females, whereas it shows a positive relationship with SMU in millennial females. Thus, the study has yielded meaningful relationships between the big five personality traits and SMU across the generations.

Keywords: Indian youth, Gen Z population, millennials, personality factors, social media usage, attitude towards technology

Dimple Kariya and Geetika Tankha

INTRODUCTION

Rushk off (2019)has rightly commented to "The human, "there's no u-turn to achieve the pre-digital era:"We can't go back; we must go through."Even folks that were anti-internet inferred that social media is inevitable and protracted. The current study revolved around studying the relationship between personality traits and social media usage between the following two generations, i.e., Millennials who were born between the year1981-1996 and the age range would be 26 – 41 years currently, and the Gen Z population who were born between the year 1997 – 2012 and their age range would be 10 – 25years. Social media usage has become one of the critical tools for communication in today's times, and observing the rapid growth of technology. Curiosity emerged to learn and understand how the above two generations have adapted to this revolutionary time. The Millennials were the ones who have witnessed the times without technology, to the introduction of it slowly and then finally the full-fledged use of it, without which the lives of Gen Z are unimaginable. Gen Z is the population born amidst the boom of technology, and not only that, but they have also learned only this way to communicate since childhood. This study explored the relationship (if any) in the use of social media and the attitude towards technology among both generations with their personality traits.

Maggie Jackson (2011) explores how humankind's generation has changed and how the hustle and multitasking have taken over by adherence to non-essential tasks. Now toddlers have started spending most time glaring at the screens. They're unaware of the outside world and the current times and are mindful that this small box-like creature blows their parents. The younger generations have been taught to be half present with the modeling parents have done to them. As per an estimate, it's seen that folks tend to view their devices quite constantly to receive a dopamine rush. The book says that we have woken up to difficult times brought by ourselves where we are deep into distraction. Social media have confiscated the power of self-control and, therefore, the capacity to think critically. Critics claimed that the inventors of the famous social media sites have hacked our most crucial asset, called "attention."We can conclude that an app developer has confiscated our "cognitive liberty" and soon might require rehabilitation centers that we previously needed for drugs or alcohol addictions.

Every individual is born with only personality factors, which make them un identical to a different individual. These are innate traits and characteristics, and also the person will inevitably progress with the inherited personality factors. These factors and their impact on their lives would change with time, looking at the individual's practical experience in his life. However, nobody can remove the fundamental characteristics of their personality. As personality could be a vast and detailed topic, psychologists have tried to figure this out in a broader picture, thus segregating them into 5 factors. The "Big Five " (Goldberg,1981) was a title chosen to precise that each factor is immensely comprehensive, which 'doesn't mean to indicate that the elements are inseparable. It would be wrong to mention that these 5 factors show personality differences. Instead, they signify personality on a far broader level of understanding, and each aspect has its own specific and significant personality characteristics.

Extraversion demonstrates an individual who adopts a more social trait and can manage to communicate well with others, and knows how to create boundaries when required. Agreeableness Individuals who tend to be agreeable are more submissive to other people's needs and are very modest. Conscientiousness can be defined as behavior exhibited only after giving it a thought, well analyzed, and then executed. These individuals are very aware of what they want and are doing. They can control their urges to accomplish the critical task at hand. Neuroticism leads to one feeling anxious, lonely, sad, and tense. They are the people who experience all the negative emotions in day-to-day life. Finally, openness can be described as an individual’s flexibility to adapt to various situations with an open

mind and not become rigid when displaying judgment(Costa & McCrae,1992; John, 1990). The big five personality factors seem to have a relationship with individuals' social involvement, the amount of interaction, and how they present themselves in the social circuit (Aspendorpf & Wilpers, 1998). Due to this codependency among personality factors and individuals' involvement in the society, personality factors are used to study the use of SM and social networking sites (Ross *et al*., 2009; Amichai- Hamburger & Vinitzky, 2010) Personality traits like Extraversion mean a decently pleasant, cheerful, and outgoing person. These people could be expected to share more on social media as they would have a lot to share and learn from others, make more friends and share more mutual friends. According to Borman and Motowidle (1993), people high on extraversion would have more interpersonal interactions and be socially active by nature. Davies *et al.,* (1998) and Hill (2004) studied neuroticism and the person having the impression in interactions and found no relationship. This could mean that individuals with neuroticism were not quite self-reflective or not very open to interacting and taking the initiative with people. Similarly, Vazire and Gosling (2004) interpreted a significant relationship between agreeableness and impression management. This means that these individuals are more precise in writing in their bio and describing every detail about the incident on the SM to share with the followers or public. This means they could be one of the highest users of SM. Wilson *et al.,* (2010) studied that individuals high in conscientiousness would use more petite SM. This could come from the self-monitoring factor, a common topic of discussion these days. People with reasonable control over how much time they spend interacting with people tend to view SM usage as time wastage and therefore use it more mindfully. He also believed that Openness was not correlated to SMU. He thought those open to experiencing new incidents would be more interested in face-to-face or other forms of interaction than SM. He also believed that this could be due to the monotony that SM has brought in now, as nothing changes, so there is no curiosity.

METHOD

Sample and Procedure

A cross-sectional survey design was used in this research. The sample consisted of millennials and the Gen Z population working and studying full-time. The study used a survey design with independent variables being the age (Millennials and the Gen Z) and gender (females and males) of the Indian young adults. Purposive sampling was used for selecting the sample. The samples were selected based on the following criteria;(a) Gen Z who are aged 18-24 years; (b) Millennials who are aged 25-35 years; (c) All samples belonged to a non-engineering background. Data were collected using both online and offline surveys. The sample consisted of 392 young Indian adults. It included 200Gen Z population (61=females and 89 college-going males) in the age range of 18-24years and 192 millennials (78=females and 72 college-going males) in the age range of 25-35years. The nature and purpose of the study were explained to the participants, and their consent was taken to participate in the study. They were assured of the confidentiality of data and were free to back out from participating in the study.

Measures used

1. 1.Demographic Information Sheet**:** The demographic information includes the name (optional), age, gender, education level, residence, work, or occupation details.
2. 2.The Media and Technology Usage and Attitudes Scale (Rosen *et al*., 2013): The MTUAS is a self-report instrument that assesses information technology and social media usage (44 items) as well as attitudes toward technology (16 items) of adults. The first 40 items, regarding technology and media usage, are rated by frequency of use in a 10 point Likert Scale, and items 41 to 44 are assessed with 9 points Likert Scale. It includes 11 subscales, while the attitudes dimension consists of 4 subscales.
3. 3.The Big Five Inventory (BFI) (John & Srivastava, 1999). The questionnaire consisted of 44 items. Answers were scored on a scale ranging from 1 (strongly disagree) to 5 (strongly agree). BFI items are good descriptors of all five personality dimensions (Openness to experience, conscientiousness, neuroticism, extraversion, and Agreeableness). Internal consistency coefficients of reliability (Cronbach Alpha) on the Indian sample were good (a=0.83) .

Statistical Analysis

Descriptive statistics (Mean, Standard deviation) Pearson correlation coefficient using SPSS version 26 were conducted for the present study.

RESULTS AND DISCUSSION

The present study mainly focused on examining the relationship between the Big five personality traits: Extraversion, Agreeableness, Conscientiousness, Neuroticism, Openness, social media usage, and attitude towards technology in young adults. This study examined the relationship of personality traits in males and females as well as in the Millennials and Gen Z population. The obtained results are further discussed as they have led to interesting findings. Table 1 depicts the descriptive statistics for the four groups on SMU, attitudes towards it, and personality traits. It is observed that the SMU mean score is highest in Millennials (M= 208.61; SD=42.50) compared to all the other groups. Interestingly, the female population has a higher mean score on SMU than the males (M=199.26; S.D=42.39).A positive attitude towards technology is highest in millennials (M=4.15;S.D=0.58) compared to the other groups. Anxiety about being without technology is also highest among the millennials (M= 3.63; S.D= 0.90), followed by the female population (M= 3.44; S.D= 0.96)Negative attitudes toward technology are very high in millennials (M= 3.53; S.D=0.85), followed by female population (M= 3.52; S.D=0.87) among the four groups. This clearly states that the 25-35 years are more connected and use more social media sites than the younger population. This could be due to Cyber ostracism, which means feeling left out or isolated in the virtual world. This constant and persistent desire to be continuously liked and accepted might be the key reason behind using social media more. This can be explained by a research study by Mai and Vorderer (2015), which indicated that millennials feel insecure and constantly be present online to make sure that they are the first ones to respond or reply to the comments or messages, or emails or they keep a keen eye on who is the first one to react to their news on Facebook. Therefore, this constant fear of missing out can be the reason for the high anxiety about being without social media. This fear of missing out could, in turn, be a reason for people to have concerned about no social media. On the other hand, the Gen Z group might have scored low as they are believed to be observant and think from a broader perspective, including physical, emotional, financial, and educational, and they are very well aware of the right and wrong in the path of achieving their professional goals in the future (Chicca & Shellenbarger, 2018; Hampton & Keys,2017; Shatto & Erwin 2016).The Big five personality indicated that the millennials had scored higher on extraversion, agreeableness, and conscientiousness (M=3.42; S.D=0.65), (M=3.81;S.D=0.56), and (M=3.60;S.D=0.58) respectively, compared to all the other 3 groups. Neuroticism and Openness show the highest mean score in the females (M=3.13;S.D=0.46) and (M=3.63;S.D=0.46).

Neuroticism and Openness could be high in the female populations due to the constant apprehension about their career and the need to prove themselves at every stage. Even today, in the upper-middle class, literate and non-traditional families, females are expected to follow certain milestones in their lives, be it getting married at the right age, expanding family at the right age, etc. In this race of constantly proving their self-worth and juggling responsibilities, women might be feeling anxious all day long. Due to their expectation from society to be specific and hospitable and interact with everyone, they may make themselves open to mingling and getting to know people.

Table 2 presents the correlation coefficient between personality traits and the measures of SMU. It indicates a significantly positive relationship between agreeableness and negative attitudes toward technology (r= 0.199, p=<.01). In other words, individuals who are high on agreeableness have a negative attitude toward technology in the Gen Z population. Conscientiousness tends to have a negative relationship with a positive attitude towards SMU (r=-0.188,p=<.01) and anxiety about being without technology (r=0. 203,p=<.01). This implies that Conscientiousness leads to a negative attitude towards technology and will reduce the anxiety about no technology. A positive relationship exists between neuroticism and anxiety about being without technology (r=0.197,p<.01) and negative attitudes toward technology (r= 0.172,p<.05), respectively. This means that neuroticism leads to anxiety about no technology and a negative attitude towards technology. Openness is positively related to negative attitudes toward technology

(r=0 .183,p<.01). Openness leads to a negative attitude towards technology in Gen Z. Wilson *et al*., (2010) study concluded that those who scored higher in conscientiousness would tend to have a less positive attitude towards SM. The results of Gen Z were contradictory; they were in line with the study of Marcus *et al*. (2006), who concluded that those scoring higher in conscientiousness would tend to have a more negative attitude towards SM . These findings align with Marcus *et al*. (2006), who found that individual high in conscientiousness seek social approval via SMU. Therefore, the usage and support are directly proportional to each other. In Gen Z, Neuroticism has a positive relationship with anxiety about no SMU. The results are on the same wavelength as the literature available. It is observed that populations with high anxiety and apprehensive nature are more involved with SMU and would show signs of anxiety if they are prohibited from using SM for some reason. However, correlation analysis indicated that the millennial population high on neuroticism shows a negative attitude towards SMU, which contrasts with the results found in a study by Smith-Duff (2012),where correlation indicated a positive relationship between Facebook and SMU. Table 3 presents the correlation coefficient between personality traits and the measures of social media usage. It indicates a positive relationship between Extraversion and a positive attitude toward technology (r=0.218,p=<.05). In other words, extraversion leads to millennials' positive attitude toward technology. Conscientiousness has a negative relationship with a negative attitude towards technology(r=-0.167,p=<.05). This means that conscientiousness leads to a positive attitude toward technology. Neuroticism tends to have a positive relationship with anxiety about no technology (r=0.206,p=<.01). We can say that neuroticism increases anxiety about no technology in millennials. Openness positively correlates with a positive attitude towards technology (r=0.279,p=<.01). This means openness increases a positive attitude towards social media. The findings from this study support Landers and Lounsbury (2006) that as agreeableness increases, the negative attitude toward technology increases. In other words, disagreeable people are more prone to using SM or having a positive attitude towards technology.

The results of the above study coincide with the study by Correa *et al*.,(2010); those who are acceptable to explore new experiences in life with an open mind are more likely to explore social media sites. As per Table 4, conscientiousness has a significant negative relationship with a positive attitude toward technology (r=-0.278,p=<.01). In other words, conscientiousness increases and reduces a positive attitude towards technology. There is a negative relationship between openness and SMU(r=-0.218,p=<.05)and a negative attitude towards technology (r=-0.241,p=<.05) in Gen Z males. This means that openness reduces SMU and negative attitude toward technology. Our results were on the same lines as the previous research, which pinned down that highly conscientious individuals show less positive attitudes toward technology (Lander & Lounsbury, 2004). In the current study, we see our results on the same wavelength as that of Lampe, Ellison & Steinfeld (2007). They say people high on openness already have friends and colleagues who support them, so the motivation to use SM would be very low. They would also have a negative attitude towards technology due to the leak of data or, in other words, lack of confidentiality concerning their profiles and details.

As per Table 5, conscientiousness has a significant negative relationship with anxiety about no technology (r=-0.218,p=<.05). In other words, conscientiousness reduces anxiety when no technology is available. There is a positive relationship between neuroticism and anxiety about no technology in the millennial males (r=0.314,p=<.05). This means that men with neuroticism tend to get anxious about no SM. Openness significantly correlates with a positive attitude towards technology (r=0.243, p=<.05). The above results indicate that neuroticism leads to anxiety about no SM in males. According to Marcus *et al*.,(2006), neuroticism negatively correlates with self-monitoring. Therefore, we can say that if self-monitoring is low, individuals would not be able to control the urge to use SM. Consequently, anxiety would built up if it's unavailable. Our results were in line with that of Ciara Smith-Duff (2012),as openness indicated a positive relationship with the frequency of SMU. As per Table 6, agreeableness has a significant relationship with a negative attitude towards technology (r=0.287, p=<0.01), which tells us that agreeable females tend to have a negative attitude toward technology. Conscientiousness has a significant negative relationship with anxiety about no technology (r=-0.245,p=<.01). In other words, conscientiousness reduces anxiety when no technology is available. There is a positive relationship between neuroticism and negative attitude toward

technology in Gen Z females (r=0.216,p=<.05). This means Gen Z females with high neuroticism tend to have a negative attitude towards technology.

These results correspond with the Landers and Lounsbury (2004) study, which says that more agreeable people spend less time on SM than those who disagree .As per Wilson *et al.,* (2010), those scoring low on conscientiousness were found to spend more time on social networking sites. Anxiety will automatically increase when the usage increases if it is not provided. According to Marcus *et al*. (2006), those who were high on neuroticism could not change their behaviour and used fewer social media. This is in line with our studies where we observed those females who were high on neuroticism would have a negative attitude towards technology. As per Table 7, Extraversion has a significant positive relationship with a positive attitude toward technology (r=0.256,p=<.01). This means that extraversion increases millennial females positive attitudes toward technology. Neuroticism shows a positive relationship with SMU in millennial females (r=0.221,p=<.05).This suggests that neuroticism leads to high SMU in millennial females. Openness positively correlates with a positive attitude toward technology (r=0.322,p=<.01).This shows that openness increases positive attitudes toward technology in females. As per research, western philosophers have mentioned men being more oriented by reasoning skills and women being emotion-driven. With this characteristic, women are more open to accepting various emotions and experiences than men. Valkenburg *et al.,* (2006), show that individuals with high levels of extraversion and openness personality characteristics are more prone to SMU. They also say that millennials use SM mostly to witness and build a close bond with the social community. Our results show females with high neuroticism show higher SMU, which is contrary to the findings by Immordino-Yang, Christodoulou, and Singh (2012). This could be due to the fear of constantly missing out on the daily news coming in from the social community.

CONCLUSION

On the basis of the above findings, it can be concluded that both groups were equally influenced by the new tech era, where social media is the pulse of society. This study examined the Millennials and Gen Z population. Conscientiousness was seen to have a negative relationship with anxiety about no SM in millennial males and Gen Z population. Openness tends to have a negative relationship with SMU's positive attitude towards technology in males belonging to Gen Z and millennials. Neuroticism shows varied results in the different groups. It tends to have a positive and significant relationship with SMU in millennial females and anxiety about no technology in millennial males. This result contradicts Gen Z females, where neuroticism shows a positive relationship with a negative attitude towards SM. This tells us the difference between both generations. This could be because Gen Z, already facing anxiety due to neuroticism, might wish to stay off technology used to keep anxiety in check. Thus, the study has yielded meaningful results and added to the existing research findings in the field of personality traits and social media usage.

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Table 1. Descriptive Statistics for Millennials and Gen Z Young Adults on the study variables

Variable	Gen Z (N=200)	Millennials (N=192)	Females (N=218)		Males (N=174)	
	M (S.D)	M (S.D)	Gen Z (n=107) M(S.D)	Millennial (n=111) M(S.D)	Gen Z (n=89) M(S.D)	Millennial (n=85) M(S.D)
Media Usage						
Social media usage	186.99 (40.83)	208.61 (42.5)	186.99 (38.81)	208.70 (43.39)	183.53 (42.74)	207.99 (41.19)
Positive attitude towards technology	3.81 (0.66)	4.15 (0.58)	3.93 (0.55)	4.06 (0.56)	3.65 (0.73)	4.26 (0.58)
Anxiety about being without technology	3.22 (0.94)	3.63 (0.90)	3.32 (0.97)	3.57 (0.91)	3.09 (0.88)	3.71 (0.88)
Negative Attitudes Toward Technology	3.5 (0.85)	3.53 (0.85)	3.55 (0.87)	3.48 (0.87)	3.43 (0.81)	3.57 (0.81)
Personality Traits						
Extraversion	3.13 (0.62)	3.42 (0.65)	3.21 (0.63)	3.45 (0.72)	3.03 (0.60)	3.39 (0.55)
Agreeableness	3.61 (0.55)	3.81 (0.56)	3.65 (0.55)	3.88 (0.54)	3.57 (0.56)	3.71 (0.56)
Conscientiousness	3.22 (0.59)	3.60 (0.58)	3.13 (0.58)	3.57 (0.61)	3.33 (0.59)	3.64 (0.54)
Neuroticism	3.08 (0.66)	2.92 (0.66)	3.20 (0.63)	3.05 (0.64)	2.94 (0.67)	2.74 (0.64)
Openness	3.57 (0.47)	3.61 (0.43)	3.63 (0.46)	3.62 (0.45)	3.49 (0.45)	3.60 (0.40)

Note: SD is given in the parenthesis

Table 2. Correlation coefficients for SMU and Attitude towards technology with personality traits for the Gen Z

Variables	Extraversion	Agreeableness	Conscientiousness	Neuroticism	Openness
Social media usage	0.005	-0.089	-0.131	0.041	-0.092
Positive attitude towards technology	0.115	-0.020	-0.188**	0.008	0.012
Anxiety About Being Without Technology	-0.038	-0.124	-0.203**	0.197**	-0.039
Negative Attitudes Toward Technology	-0.017	0.199**	0.015	0.172*	0.183**

Note *p<.05.**p<.01

Table 3.Correlation coefficients for SMU and Attitude towards technology with personality traits for the Millennials

Variables	Extraversion	Agreeableness	Conscientiousness	Neuroticism	Openness
Social media usage	0.075	-0.028	-0.037	0.122	0.082
Positive attitude towards technology	0.218**	0.013	0.041	0.093	0.279**
Anxiety About Being Without Technology	0.040	-0.090	-0.118	0.206**	0.022
Negative Attitudes Toward Technology	-0.060	-0.045	-0.167*	0.023	-0.096

Note *p<.05.**p<.01

Table 4.Correlation coefficients for SMU and Attitude towards technology with personality traits for the Gen Z Males

Variables	Extraversion	Agreeableness	**Conscientiousness**	Neuroticism	Openness
Social media usage	-0.103	-0.036	-0.080	-0.097	-0.218*
Positive attitude towards technology	-0.005	- 0.135	-0.278**	0.068	-0.012
Anxiety About Being Without Technology	-0.200	-0.183	-0.109	0.196	-0.055
Negative Attitudes Toward Technology	-0.015	0.077	0.110	0.094	-0.241*

Note *p<.05.**p<.01

Table 5.Correlation coefficients for SMU and Attitude towards technology with personality traits for Millennial Males

Variables	Extraversion	Agreeableness	Conscientiousness	Neuroticism	Openness
Social media usage	-0.088	0.034	-0.017	0.003	-0.067
Positive attitude towards technology	0.191	0.115	0.150	0.161	0.243*
Anxiety About Being Without Technology	-0.125	-0.111	-0.218*	0.314*	-0.055
Negative Attitudes Toward Technology	0.005	-0.173	-0.177	0.159	-0.094

Note *p<.05.**p<.01

Table 6.Correlation coefficients for SMU and Attitude towards technology with personality traits for the Gen Z Females

Variables	Extraversion	Agreeableness	Conscientiousness	Neuroticism	Openness
Social media usage	0.077	-0.149	-0.157	-0.022	-0.009
Positive attitude towards technology	0.185	0.073	-0.003	-0.152	-0.032
Anxiety About Being Without Technology	0.043	-0.098	-0.245**	0.164	-0.063
Negative Attitudes Toward Technology	-0.036	0.287**	-0.036	0.216*	0.126

Note *p<.05.**p<.01

Table 7 .Correlation coefficients for SMU and Attitude towards technology with personality traits for the Millennial Females

Variables	Extraversion	Agreeableness	Conscientiousness	Neuroticism	Openness
Social media usage	0.160	-0.073	-0.049	0.221*	0.168
Positive attitude towards technology	0.256**	-0.022	-0.053	0.123	0.322**
Anxiety About Being Without Technology	0.140	-0.056	-0.056	0.167	0.077
Negative Attitudes Toward Technology	-0.100	0.072	-0.165	-0.042	-0.108

Note *p<.05.**p<.01

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RESEARCH ARTICLE

Convergence in the Production of Foodgrains in Indian Agriculture: An Inter-State Analysis

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ABSTRACT

The notion of 'diminishing marginal returns to capital' in the neo-classical growth theory gives impetus to the development of convergence hypothesis. Accordingly, there has been a substantial body of literature attempting to investigate the issue of convergence in the context of national and regional economies. The present research study aims to investigate the convergence hypothesis – in terms of σ-convergence and β-convergence – in the production of food grains per capita across fifteen selected Indian states over a period of 30 years from 1991-92 to 2020-21. To this end, this study makes use of traditional as well as advanced econometric techniques. Empirical results based on standard deviation, coefficient of variance, Theil's index of regional inequality and cross-sectional regression strongly support σ-divergence, indicating that inter-state disparity in terms of per capita production of food grains has increased over the years. As regards β-convergence, by applying panel unit roots tests and fixed-effects regression model, this study finds that poorer states in terms of per capita production of food grains display higher growth rates than their richer counterparts. This study, thus, finds evidence in favour of unconditional β-convergence in the production of food grains across the Indian states. The study also lends credence to the empirical finding that σ-convergence is a sufficient but not a necessary condition for the occurrence of β-convergence.

Keywords: Food grains production; σ-Convergence; β-Convergence; Theil's Index of Regional Inequality; Panel Unit Root; Fixed-Effects

INTRODUCTION

Achieving self-sufficiency in the production of food grains is widely regarded as the first step toward ensuring food security for a developing country like India. Increased availability of food grains per capita as well as its equitable distribution can effectively address the long-suffered problems of malnutrition, hunger and extreme poverty. An important aspect of India's agricultural development has been its self-sufficiency in the production of food grains. Since 1995, the country has consistently performed as a leading exporter of food grains [1]. India's total production of food grains has substantially increased from 173.88 lakh tonnes in 1991-92 to 308.65 lakh tonnes in 2020-21 [2]. This increase in the production of food grains is due to adoption of the 'New Agricultural Strategy', in 1966-67 as well as some structural adjustment programmes initiated in various parts of the country during the 1980s [3]. However, since only a few selected parts of the country have been able to adopt the new technology while other parts have suffered from stagnation, Green Revolution has exacerbated inter-regional disparities in the production of food grains and agricultural output. The empirical body of research on convergence hypothesis dates back to the seminal work of Solow [4] i.e. the neo-classical growth model. In fact, the notion of 'diminishing marginal returns to capital' in the neo-classical growth model gives impetus to the development of convergence hypothesis [5]. Since then, there has been a substantial body of literature attempting to investigate the issue of convergence in the context of national and regional economies [e.g. 6-11]. These studies have discussed two notions of the convergence hypothesis viz., 'sigma (σ) convergence' and 'beta (β) convergence'. The former implies that the cross-sectional dispersion in terms of income/output per head tends to decline over time, while the latter signifies that poorer economies/regions tend to display higher rates of growth than their richer counterparts, implying thereby a negative association between rates of growth and initial income/output. Beta (β) convergence has also been classified into Unconditional or Absolute and Conditional beta(β) convergence. The unconditional beta (β) convergence measures the relationship between growth rates of income/output per head and the initial income/output over time, regardless of the country/region-specific effects. Conditional beta (β) convergence occurs when some country/region-specific effects are included in the estimation [12]. It has also been pointed out that sigma (σ) convergence is a sufficient but not a necessary condition for the occurrence of beta (β) convergence which means that the absence of sigma (σ) convergence does not necessarily imply the absence of beta (β) convergence [13-14]. Since the last couple of decades, numerous studies have attempted to investigate the issue of convergence in the context of Indian agriculture. Chand and Chauhan [15] examine the inter-regional disparity in per capita agricultural income during 1980-81 to 1996-97 and find evidence of sigma (σ) divergence in per capita agricultural income. Mukherjee and Kuroda [16] find evidence of sigma (σ) divergence and conditional beta (β) convergence in agricultural productivity across 14 major Indian states over the period 1973-93. Ghose [17] investigate the issue of convergence in agricultural output per head, productivity of land and labour across 15 Indian states for the period 1962 to 2002 and find that sigma (σ) convergence does not hold in all the cases but absolute (unconditional) beta (β) convergence holds only in case of productivity of labour. They also find occurrence of conditional beta (β) convergence in all the cases. Somasekharan et al. [18] find no evidence of both sigma (σ) convergence and beta (β) convergence in foodgrains productivity and agricultural output across 15 states over the period 1971-2007. Mukhopadhyay and Sarkar [3] find evidence in favour of sigma (σ) convergence and both conditional and unconditional beta (β) convergences in productivity of foodgrains across 18 major Indian states. The objective of the present study is to investigate the convergence hypothesis- both in terms of sigma (σ) convergence and beta (β) convergence- in the production of foodgrains per capita across 15 selected major food grains producing Indian states over a period of 30 years from 1991-92 to 2020-21. For the purpose, the present study makes use of traditional as well as advanced econometric techniques like panel unit root and fixed effects regression model. If a conclusive finding on convergence/divergence in food grains production is arrived at based on these analyses, it will help to understand how far the 'Catching Up' hypothesis holds across the Indian states in the context of food grains production in Indian agriculture.

MATERIALS AND METHODS

The present study is based on secondary sources of information and it covers a time period of 30 years from 1991-92 to 2020-21. In order to address the objective of the study, the data on state-wise annual production of food grains have been collected from the 'Database for the Indian Economy' provided by the Reserve Bank of India (www.rbi.org). State-wise data on population for the period 1991 to 2020 have been obtained from 'Population Projections for India and States' (various issues) published by the Census of India (https://censusindia.gov.in) under the Office of the Registrar General and Census Commissioner, Ministry of Home Affairs, Govt. of India. We have selected 15 Indian states for the present study on the basis of their respective shares in the total production of food grains at all-India level. In the empirical literature, there are two key concepts of convergence hypothesis viz., sigma (σ) convergence and beta (β) convergence.

Sigma (σ) Convergence

In terms of food grains production, states are said to satisfy the condition of sigma (σ) convergence (divergence) if the dispersion of log values of per capita production of food grains across the states follows a downward (upward) trend. Standard deviation (η) and coefficient of variations (γ) are commonly used measures of dispersion. Defining $Y_{it} = \frac{x_{it}}{p_{it}}$ to denote per capita foodgrains production, where x_{it} is the total production of foodgrains in state i during time t and p_{it} is the total population of ith state in tth time, the standard deviation (η_t) and coefficient of variations (γ_t) of the logarithm of per-capita production of food grains for the year t can be defined as

$$\eta_t = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_{it} - \bar{y}_t)^2} \text{ and } \gamma_t = \frac{\eta_t}{\bar{y}_t}, \text{respectively}$$

Where $y_{it} = Ln(Y_{it})$, $\bar{y}_t = \frac{1}{n}\sum_{i=1}^{n} y_{it}$ and n is the number of states ($n = 15$ in the present study).
The Theil's index (δ_t) is a measure of regional inequality which can be defined as

$$\delta_t = \sum_{i=1}^{n} S_{it} * Ln(S_{it}/W_{it})$$

Where $S_{it} = \frac{x_{it}}{x_t}$ is the respective share of state i in the production of foodgrains during time t and x_t is the total foodgrains production at all-India level during time t. $W_{it} = \frac{p_{it}}{P_t}$ denotes the relative share of population of state i in tth time and P_t stands for the total population of India.
The regression models considered here for testing the sigma (σ) convergence are given as

$$\eta_t = \alpha_1 + \beta_1 t + \varepsilon_{1t} \quad (1)$$
$$\gamma_t = \alpha_2 + \beta_2 t + \varepsilon_{2t} \quad (2)$$
$$\delta_t = \alpha_3 + \beta_3 t + \varepsilon_{3t} \quad (3)$$

Where ε_{1t}, ε_{2t} and ε_{3t} are the usual error terms assumed to be identically and independently distributed with mean zero and constant variances. The null hypothesis (H_0) of no sigma (σ) convergence in the above three models implies that $\beta_1 = 0, \beta_2 = 0$ and $\beta_3 = 0$, while significant positive (negative) values of the coefficients indicate absolute divergence (convergence) under the alternative hypothesis (H_1).

Beta (β) Convergence and Panel Unit Root Tests

The other important concept of convergence hypothesis is beta (β) convergence which asserts that poorer states tend to catch up the richer ones in terms of food grains production. For testing beta (β) convergence, panel unit root tests have been applied. Defining $\tilde{y}_{it} = y_{it} - \bar{y}_t$ to be the deviations of y_{it} from the cross-state mean ($\bar{y}_t$) of logharithm of

per capita production of food grains for state i during the time t, the beta (β) convergence indicates that [see Evans and Karras (19)]

$$\lim_{j\to\infty} E_t\left(\tilde{y}_{i,t+j}\right) = \alpha_i \qquad (4)$$

Where α_i is the parameter that determines state i's growth path. Condition (4) holds if and only if $\tilde{y}_{it}$ is stationary for all $i = 1, 2, \dots, n$. Convergence is said to be absolute if $\alpha_i = 0$ and conditional if $\alpha_i \neq 0$. In order to check whether the series is stationary or not, panel unit root test is applied using the following equation.

$$\tilde{y}_{it} = \alpha_i + \rho\tilde{y}_{i,t-1} + u_{it} \quad \forall\, i = 1, 2, \dots, n; t = 1, 2, \dots, T \qquad (5)$$
$$where;\ u_{it} \sim iid(0, \sigma^2)$$

In case of panel unit roots, the null hypothesis is $H_0: \rho = 1$ i.e. the series is non-stationary, against the alternative hypothesis $H_1: \rho < 1$. Rejection of H_0 implies that the series $\tilde{y}_{it}$ is stationary which implies the process of convergence. Therefore, a statistically significant value of the test statistic indicates beta (β) convergence. Tests for panel unit root proposed by Levin et al. [20], Im et al. [21], Maddala and Wu [22] and Breitung [23] have been applied in this study.

Beta (β) Convergence and Panel Regression Model:

The panel regression model considered in this study for testing beta (β) convergence in the production of foodgrains across Indian states is given in equation (6)

$$\frac{1}{\tau} Ln\left(\frac{Y_{it}}{Y_{i,t-\tau}}\right) = \alpha_i + \beta LnY_{i,t-\tau} + u_{it} \qquad (6)$$

Where α_i is the unobserved state-specific effect, $Y_{i,t-\tau}$ denotes the initial production of foodgrains per capita of ith state in period $t-\tau$ and $u_{it} \sim iid(0, \sigma^2)$. This exercise has been carried out for two different choices of τ viz., $\tau = 1$ and $\tau = 5$. If the estimated value of β is found to be statistically significant and negative (positive) then beta (β) convergence (divergence) holds in case of per capita production of food grains across the Indian states.

RESULTS AND DISCUSSION

In this section, we first make a comparative analysis of the sample states in terms of their percentage shares in India's total food grains production as well as their respective shares in total population of the country. The corresponding figures are reported in Table 1. Average annual growth rates of the states in the production of food grains are also reported in Table 1. It can be observed that states like Gujarat, Haryana, Madhya Pradesh, Maharashtra and Rajasthan perform better in terms of food grains production, contributing more to India's total food grains in 2020-21 than they did in 1991-92. The remaining 9 states contribute less in 2020-21 than they did in 1991-92. It is also evident that the average shares in total food grains in states like Andhra Pradesh, Haryana, Madhya Pradesh, Punjab, Rajasthan and Uttar Pradesh are higher than the corresponding shares in total population. For states such as Bihar, Gujarat, Karnataka, Kerala, Maharashtra and Tamil Nadu, population shares outweigh their corresponding shares in total food grains production. Both population and food grains shares are very close for Assam, Orissa and West Bengal. As regards the growth performance of the states, it is observed that Tamil Nadu leads the sample states in terms of average annual growth rate, followed by Rajasthan and Gujarat with growth rates being 10.09, 8.70 and 7.82 percent, respectively, during the period 1991-92 to 2020-21. Average annual growth rate is found to be negative (-1.30 percent) in case of Kerala. States like Maharashtra, Madhya Pradesh, Orissa, Karnataka, Bihar and Haryana perform moderately with rates of growth being more than 2 percent, while states like Andhra Pradesh, Assam, Punjab, Uttar Pradesh and West Bengal are poor performing states in terms of average annual growth in food grains production.

Figure 1 gives an overview of the extent of inter-state disparities in the availability of food grains per capita per day for the year 2020-21. From the figure it can be seen that Punjab tops the rank in terms of availability of food grains of 2709.9 grams per head per day, followed by Haryana (1721.9 grams) and Madhya Pradesh (1050.3 grams), while Kerala ranks the lowest with availability of food grains of only 50.7 grams per head per day in 2020-21.

Sigma (σ) Convergence and Beta (β) Convergence

Empirical results on sigma (σ) convergence for equations (1), (2) and (3) wherein three measures of dispersion/inequality viz., Standard Deviation (η_t), Coefficient of Variation (γ_t) and Theil's Regional Inequality Index (δ_t) are taken as the dependent variables respectively and time (t) as the explanatory variable, are reported in Table 2. Results show that for all the three models, the coefficients of time variable are positive and statistically significant at 1 percent level of significance. This clearly indicates that inter-state dispersion in per capita food grains production has increased over the years. Thus, there is evidence in favour of significant sigma (σ) divergence across 15 major foodgrains producing Indian states. The diagrammatic representation of the three measures of sigma (σ) convergence is shown in Figures 2, 3 and 4. The cross-state standard deviations of the log of per capita food grains production in 15 major states during the period 1991-92 to 2020-21 are depicted in Figure 2. It can be observed that overall there has been an increase in the dispersion of per capita food grains production across the states throughout the years. The value of standard deviation has risen from 0.754 in 1991-92 to 0.882 in 2020-21. This indicates that inter-state dispersion in the production of food grains per head has increased over the years. The same conclusion can be drawn for coefficient of variation of the logarithm of per capita food grains production, which is another measure of sigma(σ) convergence. As regards the third measure of sigma (σ) convergence i.e. the Theil's index of regional inequality, it is obvious from Figure 4 that the index shows very uneven movements, with significant increasing trend followed by somewhat declining trend. The value of Theil's index has risen from 0.182 in 1991-92 to the peak of 0.262 in 2002-03 and then to 0.183 in 2020-21. Because the trend line has an upward slope, the trend has been distinctively towards greater regional inequality in terms of per capita food grains production across the major food producing states. As previously discussed, beta (β) convergence signifies that the poor performing states tend to grow faster than the high performing ones in terms of food grains production. Results of panel unit root tests for beta (β) convergence using the methodology proposed by Levin et al. [20], Im et al. [21], Maddala and Wu [22] and Breitung [23] are reported in Table 3. All the tests are performed on the demeaned series of log of per capita food grains production ($\tilde{y}_{it}$). Akaike's information criterion (AIC) is used for selection of appropriate lags in all the tests. The null hypothesis (H_0) for panel unit root test assumes the presence of unit root in the series i.e. the series $\tilde{y}_{it}$ is non-stationary. The value of the test statistic of Levin-Lin-Chu (LLC) test rejects the null hypothesis of non-stationarity of $\tilde{y}_{it}$ in favour of stationarity at 1 percent level of significance. Rejection of H_0 implies that per capita foodgrains production shows beta (β) convergence across 15 major Indian states. The same conclusion is also true for Im-Pesaran-Shin (IPS) test and Fisher Chi^2 test (Augmented Dickey-Fuller version and Phillip- Perron version) since the null hypothesis of nonstationarity of $\tilde{y}_{it}$ is rejected at 1 percent level of significance in all the tests. It is worth mentioning here that since LCC and IPS tests assume cross-sectional independence (i.e. no contemporaneous correlation across states), the finding based on these tests may raise doubt on the conclusion of beta (β) convergence in foodgrains production. To overcome this, we also perform the panel unit root test proposed by Breitung [23] which is robust to cross-sectional dependency. The value of test statistic turns out to be -4.17, which strongly rejects the null hypothesis of non-stationarity in favour of stationarity of $\tilde{y}_{it}$ at 1 percent level of significance. Considering all these tests for panel unit root, it can be inferred that the assumption of stationarity stands true for $\tilde{y}_{it}$ and there is a tendency for per capita production of foodgrains across 15 major Indian states to converge in the long run.

Empirical results on beta (β) convergence using the static panel data model are reported in Table 4. Two initial periods $\tau = 1$ and $\tau = 5$ are considered for this regression analysis. Further, to test either fixed effects or random effects model is appropriate, Hausman [27] specification test is applied under the null hypothesis that the state-specific effects ($i.e. \alpha_i$) are not correlated with the explanatory variable in the model. The value of Hausman test statistic is highly significant which rejects the null hypothesis at 1 percent significance level indicating thereby the appropriateness of fixed effects model. Modified Wald test for group-wise heteroskedasticity detects heteroskedasticity in the model, which is adjusted by using robust standard errors. From the fixed effects results, it is

evident that the beta (β) coefficient is negative (-0.582) and highly significant indicating there by a negative association between growth in production of food grains per capita and its initial level ($\tau = 1$) across the major Indian states. The same inference can be drawn for the regression with $\tau = 5$ with beta (β) being negative (-0.177) and highly significant. The results of fixed effects thus confirm beta (β) convergence in the production of food grains per capita across 15 states over the period 1991-92 to 2020-21. This clearly suggests that poorer states in terms of food grains production per head display higher growth rates than their richer counterparts and hence there is evidence that per capita production of foodgrains across the major Indian states is convergent in the long-run.

CONCLUSION

Inter-state disparity has long been a key characteristic of agriculture in India. This study is an attempt to investigate both sigma (σ) convergence and beta (β) convergence in the per capita production of food grains across 15 major Indian states during 1991-92 to 2020-21by using traditional approach as well as advanced econometric techniques. Empirical results based on conventional methods clearly indicate that inter-state disparity in terms of per capita production of food grains has increased over the years. The study thus finds evidence of sigma (σ) divergence across the major Indian states during the study period. Findings based on panel unit root and fixed effects regression model indicate that the states with lower levels of initial per capita food grains production grow faster than the states having higher initial levels of food grains production per head. There is, thus, evidence in favour of unconditional (absolute) beta (β) convergence in the production of foodgrains across fifteen major Indian states. The study also lends support to the empirical finding that sigma (σ) convergence is a sufficient but not a necessary condition for the occurrence of beta (β) convergence. The finding of the present study is significant from the standpoint of development as food security is currently receiving increased attention in the realms of national discourse and governmental policy making.

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Table 1: State-wise Share and Average Annual Growth in Production of Foodgrains (1991-92 to 2020-21)

State	Share (%) in Foodgrains in 1991-92	Share (%) in Foodgrains in 2020-21	Average Share (%) in Foodgrains (1991-92 to 2020-21)	Population Share (%) as per Census 2011	Average Annual Growth (%) in Foodgrains (1991-92 to 2020-21)
Andhra Pradesh	7.09	3.70	6.49	4.09	1.36
Assam	1.97	1.79	1.88	2.58	1.98
Bihar	6.46	5.13	5.61	8.60	2.77
Gujarat	2.00	2.88	2.68	4.99	7.82
Haryana	5.24	5.92	6.24	2.09	2.61
Karnataka	4.80	4.60	4.60	5.05	3.44
Kerala	0.63	0.21	0.33	2.76	-1.30
Madhya Pradesh	9.36	10.36	8.76	6.00	4.21
Maharashtra	5.01	5.21	5.51	9.28	5.00
Orissa	5.27	3.06	3.20	3.47	3.85
Punjab	11.32	9.65	11.38	2.29	1.55
Rajasthan	4.71	7.88	6.51	5.66	8.70
Tamil Nadu	4.88	3.71	3.53	5.96	10.09
Uttar Pradesh	21.02	18.89	19.74	16.50	1.94
West Bengal	7.48	6.51	6.96	7.54	1.68

Source: Author's estimation on the basis of RBI and Census of India data

Table 2: Estimated Regression Models for Sigma (σ) Convergence

Dependent Variable: Standard Deviation (η_t)				
Variable	Coefficient	Standard Error	t-Statistic	p-Value
Constant	0.7654	0.0140	54.75	0.000
Time	**0.0070**	0.0008	8.85	0.000
Adjusted $R^2 = 0.727$				
Dependent Variable: Coefficient of Variation (γ_t)				
Constant	0.1476	0.0033	45.23	0.000
Time	**0.0013**	0.0002	7.17	0.000

Adjusted R^2 = 0.635				
Dependent Variable: Theil's Regional Inequality Index (δ_t)				
Constant	0.1848	0.0086	21.58	0.000
Time	**0.0017**	0.0005	3.50	0.002
Adjusted R^2 = 0.280				

Source: Authors' Estimation

Table 3: Results of Panel unit Root Tests for beta (β) Convergence

Test	Value of Test Statistic	*p*-Value
Levin-Lin-Chu (LLC)	-5.6770	0.0000
Im-Pesaran-Shin (IPS)	-6.3738	0.0000
Fisher Chi^2 (ADF)	84.2439	0.0000
Fisher Chi^2 (Phillips-Perron)	188.1152	0.0000
Breitung	-4.1657	0.0000

Source: Authors' Estimation; H_0: Panels contain unit roots

Table 4: Beta (β) Convergence under Fixed-Effects Regression

Dependent Variable: $LnY_{it} - LnY_{i,t-1}$				
Variable	Coefficient	Standard Error	t-Statistic	*p*-Value
Constant	3.023789	0.6422566	4.71	0.000
$LnY_{i,t-1}$	**-0.5815606**	0.1236217	-4.70	0.000
R^2 = 0.2977 (within)				
Test for Heteroskedasticity: Chi^2 (15) = 5352.11; Prob. > Chi^2 = 0.000				
Hausman Test: Chi^2 (1) = 175.29; Prob. > Chi^2 = 0.000				
Dependent Variable: $\left(\frac{1}{5}\right) * [LnY_{it} - LnY_{i,t-5}]$				
Constant	0.9161932	0.11578	7.91	0.000
$LnY_{i,t-5}$	**-0.1766727**	0.0223015	-7.92	0.000
R^2 = 0.4553 (within)				
Test for Heteroskedasticity: Chi^2 (15) =11343.02; Prob. > Chi^2 = 0.000				
Hausman Test: Chi^2 (1) = 327.62; Prob. > Chi^2 = 0.000				

Source: Authors' Estimation

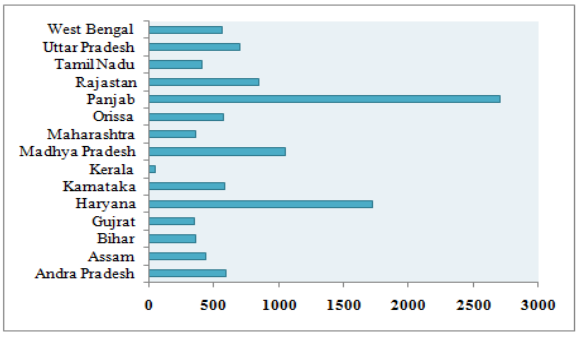

Figure 1: State-wise per Capita per Day Availability of Foodgrains in Grams in 2020-21

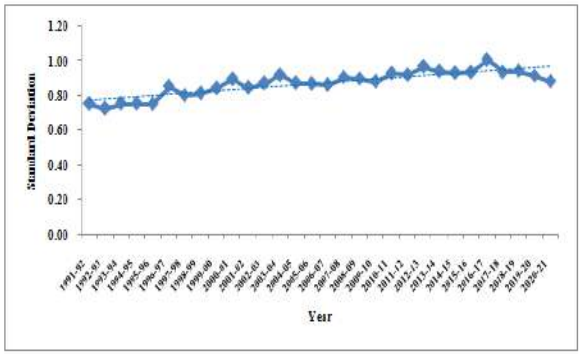

Figure 2: σ-Convergence Based on Standard Deviation across Indian States

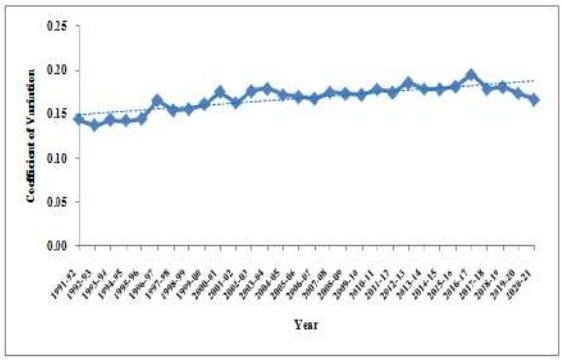

Figure 3: σ-Convergence Based on Coefficient of Variation in Indian States

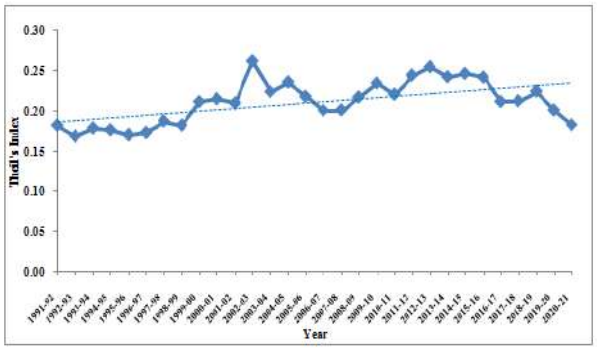

Figure 4: σ-Convergence Based on Theil's Regional Inequality Index

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RESEARCH ARTICLE

Deployment of A MEMS -based System for Detecting Health - Related Environmental Variables and Measuring Intracranial Pressure

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ABSTRACT

An excessive buildup of cerebrospinal fluid (CSF), also known as "water in the brain," is a medical condition known as hydrocephalus that causes an unnatural increase in intracranial pressure (ICP). ICP side effects include headache, blood pressure increase, shallow breathing, nausea, and confusion. The sensitivity of a pressure sensor is purely reliant on the thickness of the silicon diaphragm used in a traditional MEMS piezoresistive pressure sensor, which uses silicon diaphragm with specific thicknesses. Therefore, in order to attain great sensitivity, a thin diaphragm is needed. Therefore, the thickness requirement can be met by making use of graphene's thinness, although non-linearity difficulties must still be taken into account.

Keywords: Cerebrospinal fluid, Intracranial pressure, MEMS pressure sensor, Piezoresistive, Sensor, Sensitivity.

INTRODUCTION

In this paper with the help of this research, a novel pressure sensor device that can measure intracranial pressure and identify ambient factors that are connected to health has been designed and examined[1]. The field of medical science is greatly impacted by contributions of this nature[2]. A tiny pressure sensor is used to assess the intracranial pressure (ICP)[3]. The sensor is based on the piezoresistive effect[4]. The piezoresistive

pressure sensor is modelled and designed using finite element analysis (FEA) and nonlinear programming optimization tools[5]. There are two main sorts of sensor sizes for toddlers and adults. The sensors are made with Micro Electro Mechanical Systems (MEMS). Calculating intracranial pressure involves finding the cerebral pressure-standard ambient pressure difference (ICP). A sophisticated and flexible MEMS pressure sensor to solid state single particle sensor was made using graphene's electromechanical properties. Intracranial pressure, or ICP, is the force that substances like cerebrospinal fluid (CSF) apply to the brain tissue inside the skull[6]. High intracranial pressure can be caused by traumatic brain injury, major artery acute ischemic stroke, intracranial bleeding, intracranial neoplasms, diffuse cerebral illnesses such meningitis and encephalitis, and acute liver failure (ICP)[7]. In addition to being known as intracranial hypertension, elevated ICP over time is also referred to as sustained ICP[3]. From pressure sensors to solid state single particle sensors, complex and versatile MEMS sensors have been made using electromechanical properties of grapheme [8]. The silicon diaphragm employed in MEMS piezoresistive pressure sensors, which have particular silicon diaphragm thicknesses, determines a pressure sensor's sensitivity only[1]. Traditional Methods and Fibreoptic Intracranial Pressure Monitors are the two basic categories into which intracranial pressure monitoring techniques fall[9]. There are several ICP monitoring techniques for traditional approaches[10]. A lumbar puncture, in which a catheter is inserted into the spinal subarachnoid space, is possibly the simplest procedure to carry out[11]. But this approach has technological and decal limitations that make it less appropriate for long-term medicinal use (for example, spinal CSF pressures often do not reflect intracranial pressure, and the method presents practical nursing problems).Clinically, the most common methods of ICP monitoring are intraventricular tubes and subarachnoid bolts [12]. By uniaxially stretching monolayer graphene membranes that are sealing air-filled cavities, the piezoresistive property of graphene has been proven [13]. Geometry, or thin rectangular cavities with suspended graphene membranes sealing the voids, was what caused the uniaxial strain [11]. Then, a pressure chamber was used to house these gadgets. The pressure differential between the air in the chamber and the air trapped beneath the graphene membrane changes as air is pumped out of the chamber [14]. The trapped air pushes up against the membrane, putting strain on it[2]. With values that are tens to hundreds of times more sensitive per membrane area than the sensitivity of traditional silicon pressure sensors, very high sensitivity has been attained for these devices [15].

MATERIALS AND METHODS

Two components of the measuring assembly—the pipeline and the sensor—are responsible for the dynamic accuracy of pressure measurements. The sensor is connected to the location where pressure must be measured via a pipeline. According to the test signals employed for that purpose, the techniques for dynamic calibration of pressure sensors can be divided into two groups: frequency techniques that utilise the harmonic pressure signal and time techniques that use a step- or pulse-shaped signal. Because the step pressure signal is well realised using the time approach with a shock tube, it is strongly advised. However, the benefits of a shock tube are less obvious for piezoresistive silicon sensors, necessitating the search for other techniques.

RESULTS AND DISCUSSIONS

To understand the real outcomes by taking the references of the research objectives different models has been designed using COMSOL 5.3 a multiphysics software, which is shown in Fig.-1 to Fig.-6 with the help of six different steps. These steps includes from substrate design to model design. After analyzing the different models it has been simulated to obtain the maximum displacement. In Fig.-7 obtained displacement has been shown through the modeling and simulation. Additanily with the help of Table-2, Induced Strain through applied pressure has been highlighted. Table-1 shows that Piezoresistive is highly sensitivity at low pressure; hence it can be used in ICP invasive monitoring. Piezoresistive based ICP have much flexibility and lighter weight compare to other pressure sensors.

CONCLUSION

Piezoresistive pressure sensors have high stress, which increases sensitivity, according to this study and analysis. On the other hand, pressure is natural yet stress directly relates to sensitivity. In this study, a device structure that can detect pressure-induced strain at extremely low pressures is described. In addition, our study has shown that it is practical to use a piezoresistive MEMS intracranial pressure sensor. Graphene is also extremely sensitive at low pressure, making it suitable for ICP intrusive monitoring. ICP made of graphene is considerably more flexible and lightweight than traditional pressure sensors. As a result, it is suggested as useful significant equipment.

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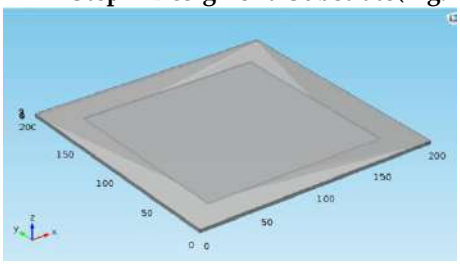
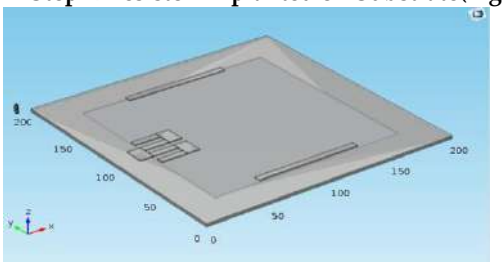
Table-1 Induced Strain through applied pressure

Type of Sensors	Pressure Applied	Strain Induced
Piezoresistive Pressure Sensor	100KPa	1.2

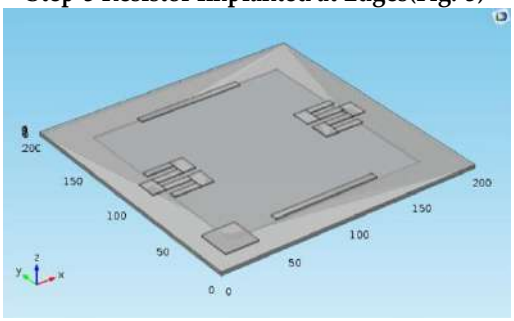
Step-1 Design of a Substrate(Fig.-1)



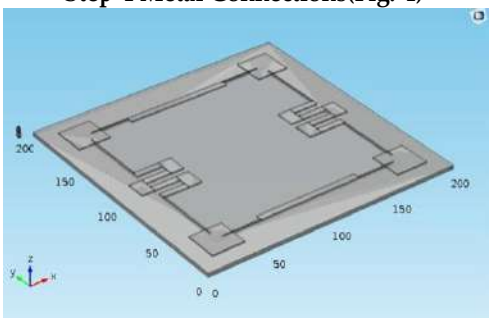
Step-2 Resistor Implanted on Substrate(Fig.-2)



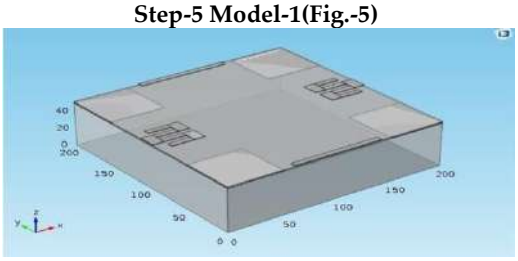
Step-3 Resistor Implanted at Edges(Fig.-3)



Step-4 Metal Connections(Fig.-4)



Step-5 Model-1(Fig.-5)



Step-6 Model-2(Fig.-6)

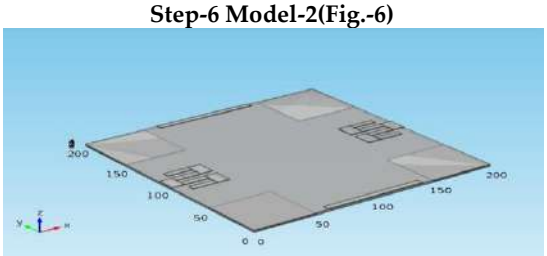


Fig:1 to 6 Modeling and Design in Multiphysics environment using COMSOL 5.3a

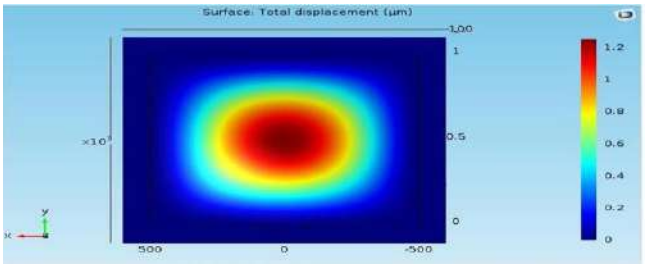


Fig.-7 Simulation of Piezoresistive Pressure Sensor

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RESEARCH ARTICLE

Interaction Effect of Liquid Formulation of *Azospirillum* and Phosphobacteria on Growth and Yield of Tomato

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ABSTRACT

Tomato is cultivated in all the states of India from Jammu & Kashmir to Tamil Nadu (North-South) and Arunachal Pradesh to Gujarat (East-West). The use of plant growth promoting rhizobacteria (PGPR) in agriculture could be a sustainable and environmentally friendly solution for crop fertilization by reducing the use of chemical fertilizers. Crop production response to bacteria with *PGPR* organism inoculants was almost equivalent to that of application of 15 - 20 kg N/ha In this study liquid formulation of *Azospirillum* and Phosphobacteria were used with eight treatments.T1- Control, T2- Recommended dose NPK,T3 -75% Nitrogen Recommended dose with *Azospirillum*,T4- 75% P_2O_5 recommended dose with Phosphobacteria,T5- 75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria,T6 - 50% Nitrogen Recommended dose with *Azospirillum*,T7- 50% P_2O_5 Recommended dose with Phosphobacteria,T8- 50% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria. The results showed that the inoculation with 75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria gives a better plant growth development. At final harvest the plant height were (121.8 cm), Leaf Area Index (3.40), Fresh weight per plant (639.22 g), Dry weight per plant (129.34 g), No of Primary Branches per plant (11.96), Stem diameter (15.90 mm), Number of fruits/plant (48.30), Yield (690.00q/ha).

Keywords: Tomato, *Azospirillum*, Phosphobacteria

INTRODUCTION

Tomato is an essential vegetable crop. It contains important minerals and vitamins needed for human health. The tomato fruit is consumed in diverse forms, as raw fruit, used as an ingredient in dishes, to make sauces, salads, and drinks. The tomato is botanically a berry fruit, it is considered a vegetable for culinary purposes. The tomato crop is cultivated in all the states of India from Jammu & Kashmir to Tamil Nadu (North-South) and Arunachal Pradesh to Gujarat (East-West). Tomato is widely produced in states like Andhra Pradesh, Karnataka, Madhya Pradesh, Orissa, Gujarat, Bihar, and West Bengal. Tomato has a more consumption rate in many developed countries and is often referred to as a luxury crop. For example, In Israel country tomato is a major part of the food basket, which is used when calculating the consumer price index (CPI). The scarcity of tomatoes can cause the Consumer Price Index to rise and influence the inflation rate. In most developing countries, tomato is becoming a more important part of the food basket. As the importance of the crop raises farmers to aim to increase the quantity and quality of the products available from the crop.

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants to uptake nutrients by beneficial interactions in the rhizosphere region of plants when applied through seed or soil. Biofertilizers accelerate certain microbial processes in the soil which increase the extent of availability of nutrients in a form that can be easily assimilated by plants. Uses of biofertilizers are one of the important components of integrated nutrient management. Biofertilizers are cost-effective and they are renewable sources of plant nutrients and are used as a supplement for chemical fertilizers. A small dose of biofertilizers is sufficient to produce desirable results because each gram of carrier of biofertilizers contains at least 10 million viable cells of a specific strain (Anandaraj and Delapierre, 2010). The employ of plant growth-promoting rhizobacteria (PGPR) in agriculture could be a sustainable and environmentally friendly solution for crop fertilization by minimizing the negative impact related to the excessive application of chemical fertilizers (Glick 2014; Ijaz *et al*., 2019, 2020). PGPR exerts a beneficial effect on plant growth through direct and indirect mechanisms. The liquid formulation is the microbial preparation that has those beneficial microbes, which have the capacity of solubilizing, fixing, or mobilizing essential plant nutrients by biological activities (Bahadur *et al*., 2016). Potash mobilizing microbes, phosphate mobilizing microbes, nitrogen-fixing microbes (NFM), and several other groups of microbes are employed in the liquid formulation (Surendra and Baby 2016). A population of *Azospirillum* (1.66×10^8 CFU/ml) in trehalose (16 mm) and phosphate solubilizing strain (3.66×10^8 CFU/ml) in the PVP (3%) is used for this formulation (Kumaresan and Reetha, 2011; Surendra and Baby 2016). Keeping these in view, the response of application of biofertilizers on growth and yield of tomato were carried out under field conditions.

MATERIALS AND METHODS

The field experiment was carried out with eight treatments, which includes two levels of nitrogen and phosphorus (75% and 50% of recommended dose i.e., 120:100:100 kg NPK per hectare) with *Azospirillum* and phosphorus solubilizing bacteria (PSB) along with control. The experiment was laid out in a completely randomized block design with three replications. Thirty eight days old seedlings were transplanted in respective experimental plots of 3X3 m^2 size at 60 X 50 cm spacing. A full dose of phosphorus in the form of single super phosphate and potassium in the form murate of potash and half dose of nitrogen in the form of urea for every treatment was applied before transplanting of crop and half dose of nitrogen were applied as top dressing after one month of transplanting.

Microbiological Parameters

The number of *Azospirillum* and Phosphobacteria were determined by the most probable number technique (MPN) technique using Döbereiner medium and Pikovaskiya s medium after incubation for 2 weeks at 37°C. All cultural operation was done in all the plots. The height of the plant was measured at 30, 45, 60, 75, 90 days after transplanting and at the time of final harvest with a meter scale. The plants were cut down after the final harvest from the ground level and weighed to get plant fresh weight. The cut plants were kept for sun drying for 3-4 days and then in the

oven at 550°C for 6-8 days. After 6-8 days of drying plant dry weight was recorded. Other parameters like the number of primary branches, stem diameter (cm), and the number of fruits per plant were observed at the final harvest. Leaf area meter was used for the measurement of leaf area index. Total fruit yield (q/ha) was calculated on the basis of per plot yield.

RESULTS AND DISCUSSION

From table I and II the significant difference was seen for plant fresh weight, plant dry weight, stem diameter, fruits per plant and yield whereas, plant height, days to final harvest and number of primary branches was not found to differ significantly.

Plant Height

There was a continuous increase in the plant height right from the transplanting to the day of harvesting but there was no significant difference among the treatments at all the stages of growth. At 30, 45, 60, 90, final harvest days after transplanting (DAT) treatment T5 showed maximum plant height (48.6, 70.6, 86.9, 96.4, 115.4, 121.8cm). *Azospirillum* inoculated tomato plants showed higher plant height than uninoculated ones (Terry *et al.,* 2000).

Leaf Area Index

It was observed that biofertilizers marginally increase the Leaf Area Index of the tomato plants in all treatments except treatment T1 (control). The highest Leaf Area Index was recorded in treatment T5 (T5 75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria). The application of *Azospirillum* increased the number of leaves in tomatoes (Mamatha and Bagyaraj, 2003). Application of Azotobacter and *Azospirillum* with 100 % N reduced the days taken to first harvest and increased the total crop duration in comparison to when these applied with 75 % N.

Fresh and Dry Weight

The least number of first harvest days was found in treatment T1 (control). Treatment T5 (75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria) was found superior for per plant fresh weight (690.00g) and per plant dry weight (129.34 g). Treatment T1 (Control) exhibited lowest fresh weight (386.50g) per plant and dry weight (98.65g) per plant. The application of biofertilizers with chemical fertilizers increased the fresh and dry weight of tomato shoot (Singh *et al.,* 2004).

Number of Branches

Highest number of primary branches (11.96) was observed with treatment T5. However, treatment T1 recorded least number of primary branches (10.35) per plant. Here, *Azospirillum* and PSB was found to increase number of primary branches when applied with 75 % Nitrogen & P_2O_5 with *Azospirillum* and Phosphobacteria Similar results were also obtained by other scientist (Gajbhiye *et al.,* 2003).

Stem diameter

Stem diameter showed wide range *i.e.,* from 15.90 to 12.90 mm. As compared to control (12.90mm), treatment T5 recorded highest stem diameter (15.90 mm *i.e.,* 15.65 % increase) followed by treatment T2 (15.50 mm). Interestingly, all biofertilizers except PSB was found to increase stem diameter when applied with full recommended dose.

Fruit Yield

Highest number of fruits per plant (48.33) was recorded with treatment T5. Application of 75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria. Highest yield (690.00 q/ha) was recorded with treatment T5 (75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria) followed by treatment T2 (642.76q/ha). Treatment T1 (control) gave lowest yield (538.98 q/ha). It was reported that when tomato seedlings inoculated with *Azotobacter* and *Azospirillum* improved crop growth and yield as compared to uninoculated ones (Sengupta *et al.,* 2002).

Effect of biofertilizers on the population of diazotrophic bacteria in the rhizosphere of tomato plants

Data in Table III obviously indicated that the population of *Azospirillum* and Phosphobacteria increased in the rhizosphere of plants inoculated with biofertilizers compared with the non - inoculated ones. The rhizosphere of plants inoculated with the mixed inoculum of *Azospirillum* and Phosphobacteria with 75 % N_2 and P_2O_5 recorded the highest population of diazotrophic bacteria. The numbers increased considerably after 30,45,60,90 days and harvest of transplanting, followed by a sharp drop. It contained 5.2, 5.5, 5.4, 5.1 cfu/g dry rhizosphere substrate for *Azospirillum* after 30,45,60,90 days and harvest of inoculation, respectively. It also, contained 5.4, 5.5, 5.9, 5.3, 5.1 cfu / g dry rhizosphere substrate for Phosphobacteria after the same intervals of inoculation, respectively.

CONCLUSION

The increase in the growth attributes and yield was steady due to the effect of biofertilizers, which fix some nutrients and make them available to the plant. On the basis of the experimental findings, application of 75% Nitrogen and P_2O_5 with *Azospirillum* and Phosphobacteria gave better plant growth and fruit yield, and also we can be saved 25 % Nitrogen and phosphate fertilizers in tomato.

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Table I Effect of biofertilizers on plant height (cm) at various stages of crop growth

Treatments	Plant Height (cm)					
	30 DAT	45 DAT	60 DAT	75 DAT	90 DAT	Final Harvest
T1 Control	44.0	68.1	82.6	92.6	109.7	112.8
T2 Recommended dose (RD) NPK	48.0	70.2	86.4	96.0	115.0	121.4
T3 75% Nitrogen RD with *Azospirillum*	46.2	69.5	85.7	94.9	113.2	118.9
T4 75% P_2O_5 RD with Phosphobacteria	45.9	69.2	85.1	94.2	112.1	117.6
T5 75% N and P_2O_5 RD with *Azospirillum* and Phosphobacteria	48.6	70.6	86.9	96.4	115.4	121.8
T6 50% N RD with *Azospirillum*	45.1	68.9	84.6	93.5	111.0	116.9
T7 50% P_2O_5 RD with Phosphobacteria	44.3	68.6	83.5	93.1	110.4	115.4
T8 50% N and P_2O_5 RD with *Azospirillum* and Phosphobacteria	46.8	69.8	86.0	95.6	114.4	120.3
S.Ed	**0.050**	**0.048**	**0.055**	**0.041**	**0.035**	**0.044**
CD at 1%	**0.15**	**0.096**	**0.117**	**0.088**	**0.074**	**0.093**

Table II Effect of biofertilizers on growth and yield of tomato

Treatments	Leaf area Index	Fresh weight/plant (g)	Dry weight/plant(g)	No. of primary branches/plant	Stem diameter (mm)	Number of fruits/plant	Yield (q/ha)
T1 Control	2.74	386.50	98.65	10.33	12.90	40.33	538.98
T2 Recommended dose (RD) NPK	3.28	598.80	127.65	11.67	15.50	47.67	642.76
T3 75% N RD +*Azospirillum*	3.05	510.45	123.50	11.00	14.94	45.00	580.00
T4 75% P_2O_5 RD +Phosphobacteria	2.95	480.65	116.78	10.33	14.46	44.33	575.76
T5 75% N and P_2O_5 + *Azospirillum* and Phosphobacteria	3.40	639.22	129.34	11.67	15.90	48.33	690.00
T6 50% N RD with *Azospirillum*	2.92	435.88	110.20	10.67	13.65	43.00	560.98
T7 50% P_2O_5 RD + Phosphobacteria	2.80	396.90	105.96	10.67	13.21	41.67	540.43
T8 50% N and P_2O_5 RD + *Azospirillum* and Phosphobacteria	3.15	558.76	125.65	11.33	15.20	46.33	596.00
S.Ed	**0.028**	**0.072**	**0.149**	**0.096**	**0.121**	**0.065**	**0.136**
CD at 1 %	**0.059**	**0.154**	**0.319**	**0.195**	**0.258**	**0.138**	**0.290**

Table III Effect of biofertilizers on the population of diazotrophic bacteria in the rhizosphere of tomato plant

Treatments	Microbial Population (×10^6 cfu/g of soil)									
	Azospirillum					Phosphobacteria				
	30DAT	45DAT	60DAT	90DAT	Final harvest	30DAT	45DAT	60DAT	90DAT	Final Harvest
T1 Control	0.6	1.0	1.3	1.1	0.8	0.7	0.9	1.0	0.8	0.5
T2 Recommended dose(RD) NPK	0.7	0.9	1.2	1.0	0.9	1.2	1.5	1.8	1.4	1.0
T3 75% N RD + *Azospirillum*	5.0	5.2	5.5	5.7	6.0	2.5	2.9	3.4	3.1	2.7
T4 75% P_2O_5 RD + Phosphobacteria	3.4	3.7	4.0	3.8	3.2	5.0	5.3	5.9	5.4	4.7
T5 75% N and P_2O_5 + *Azospirillum* +Phosphobacteria	5.2	5.5	5.9	5.4	5.1	5.4	5.5	5.9	5.3	5.1
T6 50% N RD + *Azospirillum*	4.8	5.2	5.6	5.1	4.6	3.1	3.5	3.9	3.3	2.9
T7 50% P_2O_5 RD+ Phosphobacteria	3.0	3.4	3.7	3.2	2.8	5.3	5.7	5.9	5.5	5.2
T8 50% N and P_2O_5 RD +*Azospirillum* +Phosphobacteria	5.0	5.3	5.7	5.3	4.9	5.2	5.5	5.6	5.2	4.9
S.Ed										
CD at 1 %										

A- ***Azospirillum*** **PSB-Phosphobacteria**

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RESEARCH ARTICLE

Effect of Different Spacings on Yield Parameters of Gherkin (*Cucumis anguria* L.) CV. Ajax Hybrid. Under Staking Method

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ABSTRACT

To accommodate the ever-increasing worldwide demand, India emerged as the source of the best gherkin farming, processing, and exporting. Several spacings are adopted by researchers for gherkin production, according to the following annotations. An experiment was conducted on Rabi season 2022 to find out the best spacing on gherkins at Batlagundu area near kodaikanal hills, Tamil Nadu, India to determine the effect of plant spacing on the phenological parameters of gherkin (*Cucumis anguria* L.). The experiment was included of three row spacings (30 cm, 45 cm, 60 cm) and three plant spacings (100 cm, 125 cm, 150 cm) resulting in nine treatments. Data collected from the various observations were put through to Analysis of Variance for randomized block design (RBD). The result generated from the experiment was statistically significant (P≥0.05) for all the fruit characters recorded. A wider spacing of 150 x 30 cm resulted in the greatest fruit length, girth, fruit yield per vine, and the number of fruits per vine. At narrower spacing i.e., 100 x 30 cm, increased number of days for flower initiation was found due to the increased competition among the plants due to lower space. The total fruit yield per hectare was found more in smaller spacing 100 x 30 cm as the plant population is more.

Keywords: Plant spacing, Gherkin, Growth, Fruit yield, Fruit number etc.

INTRODUCTION

Gherkin (*Cucumis anguria* L.) is a non-traditional horticulture crop that belongs to the Cucurbitaceae family that is farmed and consumed mostly in USA, Brazil, India, Africa, Brazil, Zimbabwe and Cuba (Patil et.al., 2017). Gherkins are a good source of vitamins and minerals and can be cooked, fried, stewed, or used fresh in salads (Matsumoto and Miyagi, 2012 Dzomba and Mupa, 2012 and Paramasivam *et al*., 2015). Majorly associated with other foods or processed as pickles (Bairagi *et al*., 2013 and Lima *et al*., 2006). In India, gherkin is mostly an export crop farmed in the states of Tamil Nadu, and Andhra Pradesh, Karnataka with very less or no local demand (Paramasivam *et al*., 2015). According to (APEDA, 2022) country has exported about 223,515.51 Metric tons of Cucumber & Gherkin to the world for the worth of Rs. 1,651.83 crores / 223.05 USD Millions in the year 2020-21. Fruits are high in zinc which is an essential element for the normal functioning of body tissues. It also has antioxidant properties in the battle against free radicals and has no toxicity to the animal organism. (Venturin *et al*., 2020 and Sousa *et al*., 2015). The gherkin is also recognized for its traditional therapeutic use in treating stomach aches, jaundice, hemorrhoids, and avoiding kidney stone development. (Patil *et al*., 2018). The great part of gherkin production is limited due to the lack of specific farming practices for this crop. (Morais *et al*., 2018). Plant spacing is an essential element in crop production because it allows for a more effective use of space and lowers competition among plants with similar cultural requirements. It also increases the soil's nutritional content, repels pests, will provide shade, and enhances the microclimate in terms of wind and moisture. (Aniekwe *et al*., 2015).

MATERIALS AND METHODS

This experiment was undertaken during the Rabi seasons of 2022 in the area of Viralapatti, Batlagundu, Tamil Nadu, India. The experimental location contains red sandy soil with a light grit texture, a high acidic content, a poor nutritional profile, and a low fertility status. The climate is subtropical, warm, and humid, with average rainfall and high humidity as distinguishing features. The experimental site received approximately 1480mm of rain per year, with a mean minimum temperature of 25^0C, a maximum temperature of 34^0C, and a relative humidity of 64.55 percent (Tamil Nadu agriculture weather network). The experiment was conducted on an 868 m^2 plot divided into 64 m (24 m^2) plots. The experiment was set up in a Randomized block design and three replications consisting of nine treatments with varied spacings, as shown in Table 1. Ajax hybrid Gherkin seeds are sown on January 19th, and all suggested cultural practices and plant protection measures were followed throughout the growing season. Following field preparation, seeds were planted at a rate of two seeds per hole and at the plant spacings specified to achieve the necessary population densities. The crop was supplied with 25 tons per hectare of well rotten farm yard manure along with 150 kg Nitrogen, 75 kg phosphorous and 100 kg potash as per the recommended dose of fertilizers. All P, K and 1 /4th N were applied at the time of land preparation and another 3 /4th of N in three equal parts as top dressing. The ridges and furrow irrigation method were used for surface irrigation. Observations were recorded for five different yield parameters related to Days taken to flower initiation, number of pickings, Fruit Length (cm), and Fruit girth (cm). No of fruits vine^{-1}, Fruit yield vine^{-1} (gms) and Fruit yield ha^{-1} (tons) of the plants were taken and divided in to grade wise at the harvests and recorded. The data were recorded for different characteristics were subjected to statistical analysis using Cochren and Cox (1963) method.

Data collection and analysis

Data was taken from five plants randomly selected from each plot and tagged for the purpose of collecting data.

Days taken to flower initiation

Number of days taken for the initiation of first female flower on vine was counted from date of sowing in each treatment and noted.

Number of pickings

The number of harvests harvested from the first to the last harvest throughout the crop growing season was calculated and recorded.

Fruit length

The fruits which were measured from head to tip end at marketable stage, and then the average fruit length was worked out and was expressed in centimeters.

Fruit girth

The fruits which were used to record fruit weight and fruit length were used for measuring fruit diameter with the help of Vernier caliper from the center of each fruit and average value was expressed in mm.

Number of fruits vine^{-1}

The number of fruits per vine was counted from tagged vines for each harvest of every treatment in each replication during every picking till final harvesting. They were then graded according to the weight as mentioned below and the grade wise fruit number and total number of fruits calculated and expresses.

Fruit yield vine^{-1} (g)

The graded fruits from each treatment at each harvest were weighed and pooled to arrive at grade - wise yield per vine was derived and expressed in grams as mentioned in table 1.

Fruit yield ha^{-1} (tons)

The fruit yield vine^{-1} were multiplied according to the provided plant population ha^{-1} treatment wise and expressed in tons ha^{-1}.

RESULTS AND DISCUSSION

Days taken to flower initiation

The outcome of plant spacing showed significant difference on the first flower initiation days after sowing of gherkin as shown in table 1. Maximum number of days for flower initiation i.e., 28.56 were observed in plants spaced at 100 x 30 cm respectively. The plants at 150 x 60 cm spacing took lesser number of days to first flower initiation i.e., 25.50 days respectively. This might be because greater leaf number and leaf area with wider spacing provided high amounts of assimilates in the photosynthetic process, hastening the emergence of female flowers. Similar observations were obtained by Choudhari and More (2002) in cucurbits, Devi and Gopalakrishnan (2004) in cucurbits.

Number of pickings

There was a considerable difference in the number of pickings due to the different plant spacings as mentioned in the table 1. Maximum number of pickings or fruit harvest was recorded in plants with 100 x 30 cm spacing with 11.35 pickings. The plants with spacing of 150x 60 cm recorded minimum number of pickings with 9.06 pickings. In smaller spacing the plant population is more and hence the number of grade-I fruits produced are more compared to wider spacing.

Fruit Length

Different plant spacings had significant influence on the fruit length of the Ajax hybrid in gherkin as shown in table 1. Maximum fruit length was observed in plants at 150 x 60 cm spacing with 7.06 cm. Lower fruit length was recorded in plants at 100 x 30 cm spacing with 5.46 cm. Plant population is low with broader spacing, as is competition to grow, compared to a large plant population at smaller spacing. Hence the plants acquire more nutrition, more moisture and sunlight and allow fruits to grow more in length than fruits at smaller spacings. Similar

findings were also observed by Jaffar and Wahid (2014), Sharma (2016) in cucumber, Aniekwe and Anike (2015) in cucumber.

Fruit girth

Significant differences were found in fruit girth with varied plant spacings as mentioned in table 1. Maximum fruit girth was observed in plants at 150 x 60 cm spacing with 8.96 cm in thickness. The fruits produced in plants at 100 x 30cm spacing recorded minimum fruit girth with 6.06 cm thickness. In wider spacing plant grows more with less competition so accumulates more photosynthates resulting in increased fruit length and fruit girth. Similar findings were observed by Aniekwe and Anike (2015) in cucumber, Jaffar and Wahid (2014) in cucumber.

Number of fruits vine^{-1}

There was a significant variability observed in Fruit number due to different plant spacings as shown in table 2. Maximum number of fruits vine^{-1} was observed 56.98 in 150 X 60cm spacing and minimum was observed 44.30 in 100 X 30cm. It might be due to increased availability of growth promoting components viz., nutrients, air and moisture at wider spacing. Similar findings have been reported by in cucumber Lacob *et al*. (2009), Abubaker *et al*. (2010), Mamnoie *et al*. (2014) and Oga and Umekwe (2015).

Fruit yield per vine (gms)

Different plant spacing influenced fruit yield per vine more significantly as shown in table 3. Highest fruit yield per vine was observed in plants with 150 x 60 cm spacing with 355.90 grams per vine in which 247.60 grams of fruits belong to grade 1and 60.19 grams of fruits belong to grade 2. Lowest fruit yield per vine was recorded in plants with 100 x 30 cm spacing with 263.86 grams yield vine^{-1} out of which 198.15 grams of fruits belong to grade I- and 44.46 grams fruits belong to grade II. Similar observations were also reported by Oga and Umekwe (2015), Sharma (2016) and Mamnoie *et al*. (2014).

Fruit yield per hectare (tons ha^{-1})

Varied plant spacing effected total fruit yield per hectare considerably as shown in table 4. Maximum fruit yield per hectare was recorded in plants with 100 x 30 cm spacing with 8.58 tons/ha of total fruit yield in which 6.44 tons/ha belong to grade I fruit quality. Minimum fruit yield was recorded in plants with 150 x 60 cm spacing with 4.13 tons/ha in which 2.87tons/ha of grade I fruits were produced. As the plant population is directly proportional to the fruit yield, smaller spacing plants yielded more as the plant population is more. Similar research findings were also reported by Sharma (2016), Khalid (2010) and Oga and Umekwe (2015) in cucumber.

CONCLUSION

Gherkins are pickled cucumbers that are popular across the world. They are used for pickling, cooking as a vegetable, and so forth. Different plant spacings will have varying effects on the plant's growth and output. From this research we conclude that Plants with greater spacing grew in length and width of fruit, number of fruits per vine, and total fruit production per vine and Plants with wider spacing will have less competition and will receive enough quantity of sunshine, nutrition, and water to flourish. With reduced spacing, the plants will fight for fundamental needs such as sunlight and nutrition, causing them to grow taller and thinner. So the Plants with broader spacings outperformed plants with narrower spacings in terms of fruit characteristics.

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Table 1: Fruit yield Grade wise distribution details

Grades	Fruit count kg^{-1}	Fruit weight (gms)
Grade I	150^{+}	5.00 – 8.00 g
Grade II	100^{+}	13.00 – 15.00 g
Grade III	50^{+}	17.00 – 20.00 g

Table 2: Days to flower initiation, no of pickings, Fruit length, Fruit Girth

Tr. NO	TREATMENTS	Days taken to flower initiation	No of pickings	Fruit Length (cm)	Fruit girth(cm)
T_1	100 X 30 cm	28.56	11.35	5.46	6.06
T_2	125 X 30 cm	28.19	10.59	5.61	6.57
T_3	150 X 30 cm	27.68	10.13	6.01	7.13
T_4	100 X 45 cm	28.06	10.46	5.82	6.92
T_5	125 X 45 cm	27.01	9.96	6.21	7.65
T_6	150 X 45 cm	26.03	9.40	6.69	8.11
T_7	100 X 60 cm	26.67	9.54	6.48	7.87
T_8	125 X 60 cm	25.60	9.25	6.85	8.55
T_9	150 X 60 cm	25.50	9.06	7.06	8.96
	SED	0.83	0.31	0.19	0.23
	CD (P= 0.05)	1.77	0.65	0.41	0.49

Table 3: Number of fruits per vine

Tr. NO	TREATMENTS	No of fruits vine^{-1}	Grade I	Grade II	Grade III
T_1	100 X 30 cm	44.30	39.63	3.42	1.25
T_2	125 X 30 cm	47.49	42.10	3.63	1.76
T_3	150 X 30 cm	47.82	42.70	3.27	1.85
T_4	100 X 45 cm	47.25	42.56	3.57	1.12
T_5	125 X 45 cm	50.12	44.42	3.76	1.94
T_6	150 X 45 cm	53.37	47.01	3.62	2.74
T_7	100 X 60 cm	52.49	45.87	3.94	2.68
T_8	125 X 60 cm	55.29	48.23	4.41	2.65
T_9	150 X 60 cm	56.98	49.52	4.63	2.83
	SED	3.30	1.38	0.12	0.06
	CD (P= 0.05)	1.56	2.92	0.25	0.14

Table 4: Fruit yield per vine

Tr. NO	TREATMENTS	Fruit yield vine^{-1} (gms)	Grade I	Grade II	Grade III
T_1	100 X 30 cm	263.86	198.15	44.46	21.25
T_2	125 X 30 cm	287.61	210.50	47.19	29.92
T_3	150 X 30 cm	287.46	213.50	42.51	31.45
T_4	100 X 45 cm	278.25	212.80	46.41	19.04
T_5	125 X 45 cm	303.96	222.10	48.88	32.98
T_6	150 X 45 cm	328.69	235.05	47.06	46.58
T_7	100 X 60 cm	326.13	229.35	51.22	45.56
T_8	125 X 60 cm	343.53	241.15	57.33	45.05
T_9	150 X 60 cm	355.90	247.60	60.19	48.11
	SED	9.50	6.88	1.52	1.10
	CD (P= 0.05)	20.14	14.59	3.23	2.32

Table 5: Fruit yield per hectare (tons/ha)

Tr. NO	TREATMENTS	Fruit yield ha^{-1} (tons)	Grade I	Grade II	Grade III
T_1	100 X 30 cm	8.58	6.44	1.44	0.69
T_2	125 X 30 cm	7.48	5.47	1.23	0.78
T_3	150 X 30 cm	5.75	4.27	0.85	0.63
T_4	100 X 45 cm	6.26	4.79	1.04	0.43
T_5	125 X 45 cm	5.68	4.15	0.91	0.62
T_6	150 X 45 cm	4.93	3.53	0.71	0.70
T_7	100 X 60 cm	5.71	4.01	0.90	0.80
T_8	125 X 60 cm	4.88	3.42	0.81	0.64
T_9	150 X 60 cm	4.13	2.87	0.70	0.56
	SED	0.18	0.13	0.03	0.02
	CD (P= 0.05)	0.37	0.28	0.06	0.04

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RESEARCH ARTICLE

The Potential of Functional Foods and Future Perspectives: A Review

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ABSTRACT

Let food be thy medicine and medicine be thy food," Hippocrates, the father of Western medicine, cited over 2000 years back. The concept of functional foods emerged in the 1980s that are now known to function to reduce the risk of lifestyle-related diseases. Recent studies have showcased the importance of functional foods in treating physiological conditions such as diabetes mellitus, cardiovascular ailments and immune dysfunction. The constituents of functional food are likewise demonstrated to assume a significant part in the counteraction of illnesses connected with physiological systems. The relationship between diet and health was used to demonstrate how food can help us maintain or even improve our health. The Nutritional Labeling and Education Act (NLEA) was passed in the US to provide a highly regulated foundation for ensuring the safety and quality control of these food products. Developments in research, the massive growth of the market, regulation, and quality assurance will contribute to the success or failure of the food industries across the globe. This paper gives an insight on the global market potential of functional foods and role of health claims in fulfilling healthcare objectives. The regulatory structure of functional foods in the USA has been explored and comments on the need for tighter regulations for protecting consumers from false or misleading claims are provided.

Keywords: Functional foods, Nutrition, Probiotics, Prebiotics, Conventional foods.

INTRODUCTION

Food is a substance used to sustain growth and vital processes and to furnish energy. Traditional food can be made utilitarian by expanding the strength, adding, or working on the bioavailability of a specific component. This idea of functional food will lay out claims dependent either upon expanded capabilities or decrease illness risk. The primary objective of functional foods is to "reduce the risk" rather than "prevent" it. The philosophy 'Medication and food have the same origin' which necessitates the improvement of functional food varieties.[1]Functional foods are "foods. Even though this concept is newly rising, the idea behind this concept was found in a few ancient texts from India and China. [2] Customers are all the more effectively tolerating food items with wellbeing claims to advance wellbeing, provision of fundamental supplements is prone to result in less mortality and morbidity, in this way advancing a better way of life. However, despite numerous advancements in this field, it faces two major challenges.

The first is that variation in the influence of particular dietary items on health indices has emerged from incomplete data. The lack of evidence to support the disease-diet relationship has made it difficult for the public to believe the claims made by functional food manufacturers. The second challenge is the interest of commercial companies in financial gains resulting in unsupported claims for nutritional ingredients.[3]When confronted with quickly rising medical care expenditure, the idea of functional foods is broadly remembered to have started in Japan in the last part of the 1980s. The 'low and light' category is an example of the influence of health-driven goods without a particular health claim. [4]In the future, many food products will be characterized as a functional food due to the varied effects of different components which may be related to either a state of well-being and health or to the reduction of the risk of disease. As a result, the word "functional food" should be regarded as a distinct notion rather than a single well-defined object.[5]Functional food sources are presently perceived as a particular class in Japan, and the Japanese useful food market is one of the most exceptional on the planet. FOSHUs are meals made up of functional components that impact the structure and/or function of the body and are used to maintain or manage certain health issues including gastrointestinal health, blood pressure, and blood cholesterol levels.[6]

CLASSIFICATION OF FUNCTIONAL FOODS

There are 3 classes of functional food sources in view of how these are made: ordinary food sources, modified food varieties, and food ingredients.

1. Conventional foods- these are natural, whole, unmodified food that contains bioactive components to provide health benefits. It includes fruits, vegetables, dairy, and poultry products. For example, β-glucan in oat bran lowers blood cholesterol.
2. Modified foods- these types of foods include those which are fortified, enhanced, and enriched with functional food components. For example- fruit juices modified with protein and bread enriched with folate. Biofortification is one of the recent advancements in the field of functional food which involves increasing the nutrient content of plant or animal-derived food.
3. Functional foods are foods having components that have health-promoting effects in addition to their nutritional worth. Macronutrients, critical micronutrients, and other inorganic raw materials are examples of functional dietary ingredients [7].

VARIOUS APPROACHES ARE USED TO MAKE A FOOD PRODUCT FUNCTIONAL

1.Removal of an ingredient that has been found as having an adverse consequence when eaten.

2.The grouping of a characteristic dietary part might be raised to give the ideal outcomes. Adding parts that are missing in food gives extra useful impacts.

3.In a few cases, a part, for example, a macronutrient might be supplanted as an admission of them might prompt unfavorable impacts.

4.The steadiness or bioavailability of a functional food ingredient might be expanded to lessen the sickness capability of the food.[8]

INGREDIENTS AS FUNCTIONAL FOOD CONSTITUENTS

Because of their disease-curing capabilities, the demand for novel functional foods is continuously expanding. Perhaps the most basic issue in food science and technology is finding novel functional food components from natural sources. Food industry by-products are essential because they may be used as a raw material for the creation of functional meals. In a similar context, by-products from food industries such as polysaccharides, vitamins, minerals, dietary fibers flavonoids, and bio-actives manufacture highly nutritious and functional foods.

Rice wheat, a result of the rice handling industry, represents around 10% of the all-out weight of unpalatable rice. Rice wheat is high in nutrients, minerals, fundamental unsaturated fats, dietary fiber, and different sterols. Oryzanol has been connected to an assortment of medical advantages, including a hypolipidemic impact, and hypothalamic effect. Oryzanols have various synthetic and physiological impacts, including going about as a cancer prevention agent and diminishing blood cholesterol. Oryzanol has been demonstrated to lessen cancer advancement, growth improvement in cancer-bearing mice by actuating natural killer (NK) activity as well as valuable in bringing down blood cholesterol levels. A by-product of the dairy industry- WHEY, includes a variety of valuable components, including solvent proteins such as -lacto globulin, lactoferrin, and lactoperoxidase. whey products have beneficial nutritional (e.g., high content of essential amino acids), functional (e.g., gelation, foaming, and emulsifying agent), and biological (e.g., antimicrobial, anticarcinogenic, and immunomodulatory activities) properties for health.

FUNCTIONAL FOODS MARKET OVERVIEW

In 2021 the functional foods market across the globe was valued at USD 98.9 billion and it is expected to cross USD 137.1 billion by 2026 at a CAGR of 6.8%. growth in this segment is contributed by increased awareness of health and also the rise in demand for beverages and fortified food may drive this market segment. Two of the most emerging functional food ingredients include prebiotics and probiotics. Significant investments are required for the R&D of a strain to be utilized for the development of new functional foods like probiotics. For developing a probiotic one of the main requirements that should be fulfilled is that the products should contain a sufficient quantity of microorganisms up to the expiry date. The development of these probiotics requires highly sterilized and technical equipment for the extraction and manufacturing of functional food ingredients. Hence, higher costs of production lead to higher prices of the products. Higher prices prevent consumers from buying the products even though they are aware of the health benefits.

Energy drinks that contain ingredients such as proteins, vitamins, and minerals are used by athletes for specific health benefits such as boosting energy and improving heart health and immunity. Sports drinks containing creatinine and amino acids have become popular among athletes. The energy drinks segment is expected to develop at the highest CAGR in the upcoming years. The increased acceptance of the concept of veganism has tremendously increased the consumer demand and a shift from meat and dairy products to plan-based functional food ingredients has been noticed. This also leads to the increased use of functional food ingredients in dairy products, energy drinks and infant foods, thus owing to high growth rate of functional food ingredients. This sector of the functional food market in South America is projected to develop at an exceptionally high CAGR from 2021 to 2026.

However, the non availability of raw materials and also higher production costs may challenge the growth of the functional food market. During the COVID-19 pandemic, there was restricted accessibility of significant nutrients and food production security turned out to be vital. New technologies and advancements such as nanoencapsulation, bio encapsulation, and liposomal methods are utilized for manufacturing several ingredients of functional foods such as probiotics. These advancements help in the taste and odor enhancement of food products and also offer numerous health benefits.[9]

REGULATION OF FUNCTIONAL FOOD IN THE USA

Industries that intend to develop food products to be marketed in the United States should be aware of the latest research on the health benefits of ingredients that are used in the food products. These companies should determine what health claims are made about the product that is to be marketed and also whether those claims regarding the functional foods are allowed. The Nutraceuticals Research and Education Act (NREA) was introduced in Congress on November 1, 1999, but has not yet been acted on. Since congress did not provide any legal guidance, the Food and Drug Administration agency has been left with the task of defining this class of foods, and it has stated its decision that the existing regulations for foods are sufficient. Functional foods dominate the food industry in the United States for the past decade. For the success of the marketing, these products should be highly regulated for ensuring safety and quality control of these food products. The Nutritional Labeling and Education Act (NLEA) of 1990 was enacted as a result of this. After the passage of NLEA, several major changes took place in food regulations, including the passage of the Dietary supplement health and education act of 1994 and the FDA modernization act of 1997. Such regulatory developments have provided a bedrock for marketing functional foods and nutraceuticals in the United States. One of the major concerns faced by the regulatory authorities is the safety and quality control of products. Controlling these products is very essential to provide maximum benefits and health risks to the consumers. [11]Except if they incorporate "non-GRAS (Generally Recognized as Safe)" medications or mixtures that require pre-market endorsement, these things can be sold without the FDA's consent. The FDA doesn't check or assess the security of functional foods and dietary supplements in most of the circumstances. All things considered, the FDA relies upon post-market observing by gatherings, for example, buyers, medical services experts, and the Federal Trade Commission (FTC) [12].

Health-Related Claims

The relationship established between a food substance and a health condition or disease is termed as a health-related claim. Whenever a manufacturer intends to make a health claim relating to a product, the claims should be FDA approved before the marketing can commence. Health-related claims must not claim to identify a product as being able to prevent, treat, cure, mitigate or diagnose any disease; i.e., “drug claims”. The intended use of the food which is stated on the label accompanying the product solely determines the regulatory status of the product. Information provided on the labels includes total nutrition contents, nutrient claims, and some health-related claims. FDA oversees the type of health claims that may be used in the labelling of functional foods or dietary supplements in three ways.

1) FDA is assisted by the Nutrition Labelling and Education Act (NLEA) to give guidelines and to approve wellbeing claims for functional foods and dietary supplements in the wake of assessing the logical proof of the case.

2) The Food and Drug Administration Modernization Act (FDAMA) is liable for general wellbeing insurance and nutrition exploration, and it licenses well-being claims to be made in view of a legitimate statement from the National Academy of Sciences or a logical association inside the United States. These cases can be used following 120 days have passed after FDA gets a warning.

3) FDA examines applications for qualified wellbeing claims where the quality and amount of logical proof misses the mark regarding what is important for FDA to give an empowering guideline. On the off chance that the proof supporting the proposed guarantee is trustworthy and the case can be able to not misdirect purchasers, FDA gives a letter of implementation depicting the passing language that ought to go with the case and the conditions under which it expects to practice enforcement discretion for utilization of the claims in food labels.

Qualified Health Claims

At the point when new proof has been laid out between the food part and the illness decrease or any ailment, however, no critical proof is laid out for FDA to give an approval, a certified wellbeing claim might be utilized to plead FDA to permit the utilization of such a case in the labeling of food items. After FDA evaluates the strength of

the evidence and finds the evidence is credible, it issues a letter that states the conditions under which claim can be used on the food labels. Regardless of the fact that FDA enforcement discretion letters are delivered to the petitioner who sought the qualified health claim, the qualified claims can be placed on any product or dietary supplement that fits the letter's enforcement discretion conditions.

Nutrient Content Claims

Nutrient claims either determine the amount of a supplement in the item (utilizing phrases like free, high, and low) or look at the level of a supplement in one thing to that of another (utilizing terms like more, diminished, and light). The standards controlling the utilization of Nutrient content claims are intended to guarantee that distinct terms, for example, "high" and "low" are utilized reliably across all food items and are consequently significant to purchasers. As determined in the regulation permitting its utilization, healthy is a nutritional substance claim that distinguishes food as having fat-free, immersed fat, cholesterol, and salt levels.

Structure/Function Claims

Conventional foods, nutritional supplements, and drugs have long included structure/capability declarations on their labels. The Dietary Supplement Health and Education Act of 1994 (DSHEA) laid out various explicit administrative models and strategies for laying out these cases, as well as two sorts of dietary enhancement marking claims: general well-being claims and nutrient deficient disease claims. The job of a supplement or dietary component in impacting the typical construction or capability of the human body is made sense of by a structure/capability claim, for example, "Calcium makes solid bones". They may likewise portray how a nutrient or dietary component assists with saving such construction or capability, for example, "fiber assists with keeping up with gut routineness" or "cell reinforcements help to keep up with cell integrity The manufacturer should have confirmation that the case is true and not deluding, and the manufacturer should illuminate FDA with the claim's assertion no later than 30 days subsequent to showcasing the dietary supplement with the claim. If a dietary supplement label makes such a claim, the name should incorporate a "disclaimer" making note of the claim that it has not been endorsed by the FDA.

Ingredient Considerations

The federal government establishes safety standards that prohibit any food product from being adulterated. Food products containing any poisonous or deleterious substance in amounts that may be harmful to the health is said to be adulterated. A food product may be adulterated by many ways some of which include introduction of filth, unapproved pesticides or radiation. Food additives used in food products before January 1, 1958, and dietary components used in food products prior to October 15, 1994, are excluded from the definition of novel ingredients. Any ingredients that were introduced after these dates are termed as “new ingredients” and should be approved legally prior to their marketing. The notification procedure, requires the necessary information to be submitted to FDA which should support the fact that food ingredient will be reasonably safe. This certification of ingredients by competent persons based on scientific methods and testing the is critical for a product to be widely accepted as safe.

Labelling of foods and beverages

Under the arrangements of the Federal Food, Drug, and Cosmetic Act, the United States Food and Drug Administration (FDA) firmly manages and directs labelling. The Dietary Supplement Health and Education Act (DSHEA), presented in 1994, upholds the FD&C Act with new dietary supplement explicit requirements. Despite the fact that there is no legal definition for "functional foods" or "nutraceuticals" under the FD&C Act or related parts, the FDA perceives that the two classifications are covered by the act.[13]

CONCLUSION

Functional foods have given rise to a new way of thinking about nutrition and food science. These products are getting a lot of traction because of the health advantages they provide. When increasing research efforts to find

qualities and prospective uses of nutraceutical compounds are combined with the public interest and customer demand, functional foods and nutraceuticals continue to rise constantly. Establishing safety and efficacy through human proof-of-concept research and animal studies gives functional foods legitimacy, which raises consumer awareness. Increased market penetration of functional foods can be attributed to the inclusion of health claims. Consumers embrace food items that offer health advantages beyond providing required nutrients, and they are more likely to result in lower morbidity and mortality, as well as improved quality of life.

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Table 1: Comparison between Conventional food, Functional foods, and Nutraceuticals

Conventional FOOD	FUNCTIONAL FOOD	NUTRACEUTICALS
Food is a material made up mostly of protein, glucose, fat, and other nutrients that are consumed by an organism in order to maintain development and critical activities as well as provide energy.	Functional foods are foods that contain substances that have health-promoting characteristics in addition to their typical nutritional value.	a food (as a fortified food or a dietary supplement) that, in addition to its fundamental nutritional content, is believed to deliver health or medical advantages.
Natural, whole-food components rich in key elements including vitamins, minerals, antioxidants, and heart-healthy fats make up conventional meals.	Bioactive chemicals present in foods are naturally found in functional foods.	Bioactive chemicals found in fortified foods, nutritional supplements, and herbal preparations are known as nutraceuticals.
Examples of conventional functional foods: Fruits: berries, kiwi, pears, peaches. Vegetables: broccoli, cauliflower, kale. Nuts: almonds, cashews, pistachios. Seeds: chia seeds, flax seeds, hemp seeds, pumpkin seeds.	Foods enriched with vitamins, minerals, probiotics, or fibre are some examples. Fruits, vegetables, nuts, seeds, and grains are all high in nutrients.	Ginseng, Echinacea, green tea, glucosamine, omega-3, lutein, folic acid, and cod liver oil are some the examples of nutraceuticals.
Natural	Natural	Nutraceuticals can be either natural or synthetic and may be available as pills, capsules, or liquids.
Traditional nutrients such as vitamins and minerals, as well as bioactive substances, are included.	Functional foods contain bioactive substances that are distinct from standard nutrition.	nutraceuticals include traditional nutrients
The FDA oversees all food produced in the United States, with the exception of meat, poultry, and egg products, which are overseen by the Food Safety and Inspection Service (FSIS).	Functional foods are regulated by the FDA through claims on food labels. Products intended for use as medications and those designed for use as food are classified differently under the US regulatory framework.	Nutraceuticals are regulated by the Food and Drug Administration (FDA) in the United States. Manufacturers must ensure that the information on the product label is accurate and not misleading, but they are not required to register their goods with the FDA or get FDA clearance prior to manufacturing or selling nutraceuticals.

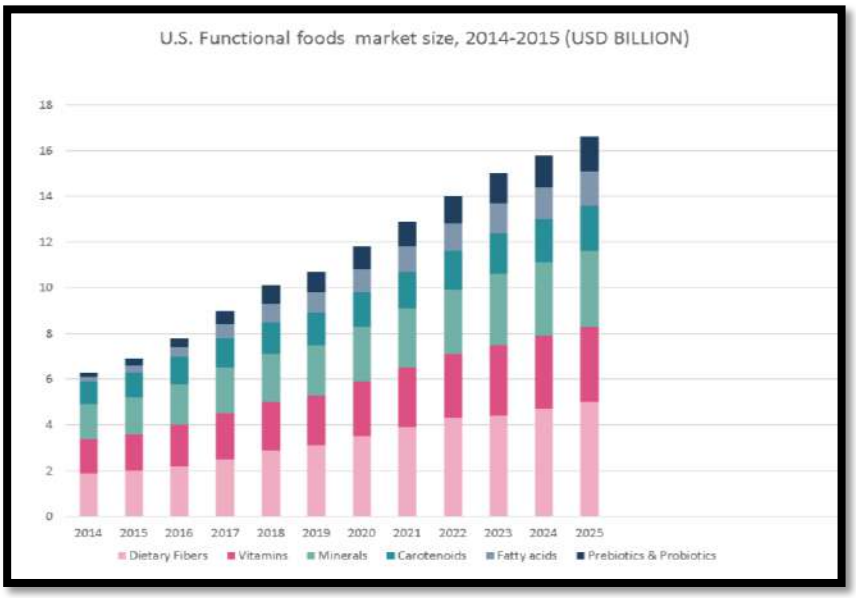

Figure 1: U.S Functional food market (2014-2025)

REVIEW ARTICLE

Scoping Antiviral and Immunomodulatory Activity of Siddha Polyherbal Formulation *'Arathai Kudineer Churnam'* with Special Mention to COVID-19

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ABSTRACT

The health care system in India is still struggling with the second wave of COVID-19 which is getting worse day by day. The role of immunology to deal with this unprecedented pandemic has been a stimulating concept & widespread efforts have been made to identify agents as prophylactic or therapeutic regimens to combat Covid infection or to reinforce the immune competence of the host. *Siddha* system has a lot to offer in its management because it is one of the best possible strategies to tackle the disease as it has the lowest possible side effects when compared to other forms of drugs available to treat the disease. The use of herbs for improving the natural resistance of the body against common infections has been a guiding principle of Siddha. This review emphasizes the potential role of Siddha polyherbal formulation *'Arathai kudineer churnam'* which is used to treat immuno-compromised disorders such as fever, respiratory infection, arthritis, & eczema, etc. *Siddha* equates symptoms of covid infection with *Kabasuram* which progresses in later stages as *Kabavatham, & Sannipathasuram* respectively. By analyzing the humoral theory, it is pertinent to note that, all the drugs in the formulation can pacify the vitiated *Kaba*&*Vatha* humour. Many of the bioactive principles present in the herbs are proven to be effective immune-modulators, antivirals, antioxidants, & anti-inflammatory agents based on the pharmacokinetics of their bioactive compounds. Active principles like Glycyrrhizin, Phenylpropanoid, Pipernic acid, & Baccatin III showed remarkable inhibitory activity against SARS-CoV2 in molecular

docking studies. Diary terpenoids in galangal were proved to have broad-spectrum Antiviral activity, Immunomodulatory, & inhibitory activity on pre-inflammatory mediators. It is scientifically reported to inhibit the replication of HIV. This study mainly aimed to review the Potential of *'Arathai kudineer churnam'* as an Anti-viral & Immunomodulator drug, *Siddha* concept of its herbal properties, & possible beneficial effects for prevention & management of SARS-CoV-2. Therefore, the review throws limelight to carried out further scientific studies to explore the characteristics of this formulation & develop it as a viable therapeutic alternative against Covid-19 infection.

Keywords: Arathai kudineer churnam, COVID-19, Pharmacological activities, Siddha formulation

INTRODUCTION

The immune system is a host defense system comprising many biological structures & processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, known as pathogens, viruses, & parasitic worms, & distinguish them from the organism's healthy tissue [1]. The role of immunology to treat various diseases has been a stimulating idea in the field of extensive research studies worldwide & showed an evolving opportunity in the prevention & management of disorders, & inflammatory reactions of different parts of the human body.

Effect of immunomodulatory agents

An immunomodulator can be defined as a substance, which can influence any constituent or function of the immune system in a specific or nonspecific manner including either innate or adaptive arms of the immune response. Some of these substances, such as granulocyte colony-stimulating factor (G-CSF), interferons, imiquimod & cellular membrane fractions from bacteria, IL-2, IL-7, IL-12, various chemokines, synthetic cytosine phosphate-guanosine (CpG), oligodeoxynucleotides & glucans are currently being investigated extensively in clinical & preclinical studies [2]. Immunomodulators are grouped into three main classes: immunosuppressants, immunostimulants, & immunoadjuvants, & their applications in medicine & pharma industries as for stimulation & suppression of the immune system & used as both prodrugs & prophylactic drugs for the healthy populace [3]. In addition, the immunomodulators from the plant kingdom seem to be a good substitute for synthetic chemical compounds [4]. It can enhance or inhibit the immunological responsiveness of an organism by interfering with its regulatory mechanisms. These may be antigen-independent & may directly induce production & effector molecules by the immunocompetent cells. These may selectively activate either cell-mediated or humoral immunity by stimulating either T-helper cells (1 or 2) type of cell response respectively [5]. World Health Organization has put a public health emergency by putting COVID-19 as a transnational threat [6,7]. It is a highly virulent viral infection with an incubation period ranging from2-14 days & the mode of transmission is by breathing of infected droplets or contact with infected droplets. Coronavirus belongs to the family Coronaviridae [8]. The number of cases recorded daily is already more than twice compared that during the peak of the first wave. but on the safer side, Vaccines are effective around the world, including in India. Most of the symptoms that existed during the first wave are still prevalent like fever, myalgia, dry cough, fatigue, & loss of smell or taste. Besides this, there are newer symptoms of the second wave of COVID-19 infection like headache GI tract infections, loss of hunger, diarrhoea, vomiting, abdominal pain, hearing loss, extreme lethargyor weakness, pink eye/conjunctivitis, dry mouth, skin rashes. Old age people or those who have chronic conditions like diabetes, heart disease, lung disease, or who have compromised immune systems may be at higher risk of serious illness [9]. Even in this COVID -19 pandemic, the role of immunomodulators is well-established as a key component. Conventional immunomodulatory chemotherapy costs a huge expense & it is unaffordable. Many synthetic drugs are being used in immunotherapeutics & the adverse side effects caused by them have produced awareness to limit their usage & to search for safe alternatives. So, there is a need for an alternative safe, cost-effective immunomodulator. In recent times, there is a surge among people in choosing alternative systems of medicine especially *Siddha*, over modern

medicine for their treatment aspects. *Siddha* system has a lot to offer in the management of immunocompromised disorders which has the lowest possible side effects when compared to other forms of drugs available to treat disease. Certain herbal drugs are believed to promote positive health & maintain natural resistance against infection by re-establishing body equilibrium & conditioning the body tissues. A wide number of herbal preparations/formulations have been indicated in Siddha literature for the management of several diseases that result in immune deficiency. The Siddha system has tested thoroughly the herbs & the polyherbal formulations in vitro & in vivo which include the *Uraimathirai*, *Saya chooranam*, *Nilavembu kudineer*, etc [10-12]. *Arathi kudineer churnam* (AKC) is a polyherbal formulation, it contains five plants namely *Alpinia galanga* (L.) Willd. *Abies webbiana* (Wall. ex D. Don) Lindl, *Alpinia officinarum* Hance, *Glycyrrhiza glabra* L, *Piper longum* L. in equal partsbased on the Siddha Text (SFI 2014). Both the varieties of Arathi (Alpinia galangal & Alpinia officinarum) have been used in this formulation & it is used in treating immunocompromised disorders such as eczema, arthritis, respiratory infections, Dropsy, & fever [13]. In addition, the bioactive components reported in the plant possess various therapeutic activities like antiviral, antioxidant, & anti-inflammatory properties against many kinds of viral pathogens. This article aims to bring out the potential role of *'Arathai Kudineer churnam'* as an anti-viral & immunomodulator drug, the Siddha concept of its herbal properties, & the possible beneficial effect for the prevention & management of SARS-CoV-2. The scientific details including morphological description, phytochemical constituents, & their pharmacological studies were collected from different literature & available published journals. However, further scientific studies & data are required to support the use of this formulation.

Concept of immunomodulation in Siddha

Great Tamil saint *'Thiruvalluvar'* has insisted on the significance of diet as the cause of disease in his chapter called *Marunthu* (Medicine). As per the *Siddha* system "Food is medicine & medicine is food" indicates that a proper diet & a healthy lifestyle containing medicinal herbs are intrinsic elements that allow the body to remain healthy [14]. Immunity is coined as '*Vanmai'* in *Siddha*& it has a direct relationship with Uyir thathukkal (*Vali, Azhal, Aiyam*) & Seven Udal thathukkal (Body tissues). The human beings are the subtotal of Uyir thathukkal & Udalthathukkal. Natural immunity of the human body by birth is called *Iyarkai Vanmai*, its improvement with the help of intake of balanced food & medicines is called *Seyarkai Vanmai* & *Kala vanmai*, which is further defined as the change of physical state under the effects of seasons & in their affected state there might be possibilities of disease [15-16]. A person is called healthy, if he possesses an equilibrium state of the doshas (body humors), Agni (bio-digestive fire), dhatus (tissues), & malas (waste products of the body) associated with a pleasant state of soul, sensory organs & mind. It is the basis for normal immunity. Disequilibrium or derangement of doshas etc. causes diseases [17-20]. The body is firmly supported by *vatham*, *pitham*, & *kapham*. Abnormal & vitiated *vatham* causes derangement of the immune system that produces diseases. Hence Siddha states that this dosha is the prime because it controls all body systems. Agni is represented as *pitham* in the body. Abnormal & vitiated *pitham* greatly disturbs digestion & metabolism leading to the development of diseases. Abnormal & vitiation of *kapham* greatly alter the immune system resulting in disease. A diminished state of doshas is not capable of vitiating other dhatus. A total of seven dhatu (tissues) are mentioned in the *Siddha*i.e, *saaram*, *chenneer, oon*, *koluppu*, *enpu, moolai, & Sukkilam* / *suronitham*. All the above seven dhatu support & nourish the body. Whenever the expression or function of one of this dhatu is impaired, an immune disturbance occurs & develops disease. Proper elimination of the malas indicates good health, & any abnormality is the cause of disease development [17-20].

***Arathai kudineer churnam* – Pharmcognostic aspect :**

Botanical aspects of herbs found in AKC were mentioned below.

1. **Tamil name** **:** Perarathai
   Synonyms **:** *Alpinia rheedei* Wight, *Alpinia viridiflora* Griff.
   Botanical Name **:** *Alpinia galanga Linn.*
   Family **:**Zingiberaceae

Morphological Description: It is a perennial, aromatic, & rhizomatous herb that is widely distributed in India. Leaves are oblong-lanceolate, acute, glabrous, green above, paler beneath, with slightly callus white margins, sheaths

are long &glabrous, & ligule are short & rounded. Flowers greenish-white, in dense flowered, 30 cm Panicles; bracts ovate-lanceolate. Calyx tubular, irregularly 3-toothed. Corolla lobes oblong, claw green, blade white, striated with red, rather more than 1 cm long, broadly elliptic, shortly 2-lobed at the apex, with a pair of subulate glands at the base of the apex, with a pair of subulate glands at the base of the claw. Fruit the size of a small cherry, orange-red [21].

Part used: Rhizome

Chemical constituents

Alpinia galangal contains essential oil pinene, b-pinene, limonene, aterpineol, linalool, methyl eugenol, eugenol & 1, 8-cineol. It contains phytoconstituents such as quercetin, kaempferol, isorhamnetin, kaempferide, quercetin, galangin, galangal A, B &galanolactone ,Methyl cinnamate, p-methane-1,8-epoxy-acethoxychavicol acetate alpinin, kaempferide, 3-dioxy 4-methoxy flavone, pinene, camphor,pineol, galangin, (1R,3S,4S)-trans-3-hydroxy-1,kaempferol, kaempferol-4'-methylether, methylcinnamate, kaempferol-7'- methylether, methyleugenol, α-thujene, α-pinene, 3-carene, βpinene, camphene, myrcene, p-cymene, borneol, α-terpineol, 4- terpineol, fenchyl acetate, bornyl acetate, α-humulene, zerumbone[22] .

Mechanisms of Action: Alpinia

contains chemicals that block certain Steps in the Swelling (inflammation) Pathway. The gingerols & diarylheptanoids constituents of alpinia are potent inhibitors of PG synthetase (prostaglandin biosynthesizing enzyme). Diarylheptanoids contained in *Alpinia oxyphylla* down-regulate cyclooxygenase-2 &iNOS expression through suppression of NF-kappaB activation in the TPA-treated mouse skin [23].1'S-1'-Acetoxychavicol acetate from the rhizomes of *Alpinia galanga* has shown a potent inhibitory effect on the production of nitric oxide in lipopolysaccharide-activated mouse peritoneal macrophages [24].

Drug-Botanical Interactions:

Alpinia may Increase Stomach Acid, & thus may decrease the Effectiveness of Antacids, Including H2-blockers. Alpinia may also interact with Proton Pump Inhibitor (PPIs). Side effects of Alpinia galanga: Decreased Blood Pressure, Pruritus (itching), Abnormally slow movements or alterations In Movement, Diuresis, & Prolonged Sleep Time.

2. Tamil name : Athimadhuram
Synonyms : *Glycyrrhiza alalensis X.Y. L, Glycyrrhiza brachycarpaBoiss.*
Botanical Name : *Glycyrrhiza glabra*
Family : Fabaceae

Morphological Description: *Glycyrrhiza glabra* Linn is a perennial shrub, attaining a height of up to 2.5 m. The leaves are compound, imparipinnate, & alternate, having 4-7 pairs of oblongs, elliptical or lanceolate leaflets. The flowers are narrow, typically papilionaceous, borne in axillary spikes, lavender to violet in color. The calyx is short, campanulate, with lanceolate tips & bearing glandular hairs. The fruit is a compressed legume or pod, up to 1.5 cm long, erect, glabrous, somewhat reticulately pitted, & usually contains, 3-5 brown, reniform seeds. The pieces of root break with a fibrous fracture, revealing the yellowish interior with a characteristic odor& sweet taste [25-26].

Part used: Roots

Chemical constituents: The roots of *Glycyrrhiza glabra* Linn. contain glycyrrhizin, which is a saponin that is 60 times sweeter than cane sugar; Flavonoid rich fractions include liquirtin, isoliquertin liquiritigenin, &rhamnoliquirilin& five new flavonoids- glucoliquiritinapioside, prenyllicoflavone A, shinflavanone, shinpterocarpin& 1-metho-xyphaseolin isolated from dried roots. The presence of many volatile components such as pentanol, hexanol, linalool oxide A & B, tetramethyl pyrazine, terpinen-4-ol, α-terpineol, geraniol & others in the roots is reported. Presence of propionic acid, benzoic acid, ethyl linoleate, methyl ethyl ketine, 2, 3-butanediol, furfuraldehyde, furfuryl formate, 1-methyl-2-formylpyrrole, trimethylpyrazie, maltol & any other compounds is also isolated from the essential oil.

Roots show various 2-methyliso - flavones, & an unusual coumarin, C liquocoumarin, 6 - acetyl- 5, hydroxy- 4 - methyl coumarin [27].

Mechanisms of Action

N-acetylmuramoyl peptide is glycyrrhizin analogue with potential in vitro immune-stimulating properties. Glycyrrhizic acid have been shown to inhibit growth &cytopathology of numerous RNA & DNA viruses, including hepatitis A9 & C, herpes zoster, HIV, Herpes simplex, & Glycyrrhizin & its metabolites inhibit hepatic metabolism of aldosterone & suppress 5-ßreductase, properties responsible for the well-documented pseudo aldosterone syndrome. The similarity in structure of glycyrrhetic acid to the structure of hormones secreted by the adrenal cortex accounts for the mineralocorticoid & glucocorticoid activity of glycyrrhizic acid. Liquorice constituents also exhibit steroid like anti-inflammatory activity, similar to the action of hydrocortisone.

Drug-Botanical Interactions

There is an increased likelihood of cardiac arrhythmias, particularly in individuals with ischemic heart disease, when liquorice is used in conjunction with digoxin. Estrogen-based oral contraceptives may enhance the mineralocorticoid side effects of liquorice in susceptible individuals. This may be due in part to estrogens reacting with mineralocorticoid receptors or inhibition of 11- hydroxysteroid dehydrogenase [28-31].

3. Tamil name : Thippili
Synonyms : *Piper latifolium Hunter, P. saramentosum Wall. ,*
Botanical Name : *Piper longum L.*
Family : Piperaceae

Morphological Description

It is a small shrub with a large woody root & numerous creeping, jointed stems that are thickened at the nodes. The leaves are alternate, spreading, without stipules & with blades varying greatly in size. The lowest leaves are 5–7 cm long, whereas, the uppermost are 2–3 cm long. Flowers grow in solitary spikes. The fruits, which grow in fleshy spikes 2.5–3.5 cm long & 5 mm thick, are oblong, blunt, & blackish-green. Stem: Stems numerous, 0.6 -0.9m, ascending or prostrate (not climbing) much branched, stout, cylindrical, thickened above nodes, finely pubescent [32]. Flowers are unisexual, minute, sessile bracteate without perianth very densely packed in spikateinflorescences. The male & female spikes are on separate plants. The drug has a peculiar odour & a pungent bitter taste that produces numbness on the tongue [33].

Part used: Fruit, Root

Chemical constituents

The fruit contains a large number of alkaloids & related compounds, the most abundant of which is piperine, followed by methyl piperine, pipernonaline, piperettine, asarinine, pellitorine, piperundecalidine, piperlongumine have been found in the root. The main lignans present in the fruits are sesamin, pulviatilol, &fargesin. Excluding the volatile piperine, the three major components are caryophyllene, pentadecane, bisabolene. Others include thujone, terpinolene, zingiberene, p-cymene, pmethoxyacetophenone, dihydrocarveol, & vitamins A & E. The major organic acids present are palmitic acid & tetrahydropiperic acid [34].

Mechanisms of Action

Piper longum Increases total WBC count, bone marrow cellularity, & total antibody production. It is found to activate macrophage migration index & phagocytic index, indicating immune stimulatory activity. Pipernic acid exhibits immunomodulation through the suppression of proinflammatory cytokines. Bioavailability enhancement: Piperine was found to enhance the bioavailability of structurally & therapeutically diverse drugs, possibly by modulating membrane dynamics due to its easy partitioning & increase in permeability of other drugs such as vasicine, indomethacin, diclofenac sodium etc [35,36]. It was suggested that piperine might be inducing alterations in membrane dynamics & permeation characteristics, along with induction in the synthesis of proteins associated with

cytoskeletal function, increasing the small intestine absorptive surface, thus assisting efficient permeation through the epithelial barrier [37-39].

4. **Tamil name** : Citrarathai
Synonyms : Alpinia calcarata
Botanical Name : *Alpinia officinarum Hance*
Family : Zingiberaceae

Morphological Description
Alpinia officinarum rhizome is a slightly curved & cylindrical rhizome, sometimes branched; 2.8 cm in length, 6. 15 mm in diameter; externally red-brown to dark brown with fine striped lines, greyish white nodes. The leaves are lanceolate (long & thin They can grow several feet high, with long leaves & reddish-white flowers. The rhizomes are valued for their sweet spicy flavour & aromatic scent.

Part used: Rhizome
Chemical constituents: Major chemical constituents of Alpinia officinarum include volatile oil, diaryheptanoid. sterol & flavonoids. The chemical components of this plant are galangoflavonoid,1′S-1′-acetoxy-chavicol acetate, acetoxycineoles (trans & cis)-2-and 3-acetoxy-1, 1, 8-cineoles, β-Sitosterol diglucoside (AG-7) & β-sitsteryl Arabinoside (AG-8) , phenylpropanoids (4,4′[2E,)-bis (prop-2-ene)-1, 1′-diphenyl-7, 7′-diacetate. hydroxy-1,8-cineole glucopyranosidesn& (1R, 3S, 4S)-trans-3-hydroxy-1, 8-cineole β-D-glucopyranoside [40-42]
Mechanisms of Action: Galangin helps to regulate COX & non-COX signalling pathways.

5. **Tamil name** : Thalisapathiri
Synonyms : Abies spectabilis
Botanical Name : *Abies webbiana (Wall. ex D. Don) Lindl.*
Family : Pinaceae

Morphological Description
A tall evergreen coniferous tree grows up to 60 m with strong horizontally spreading branches, young shoots covered with short brown hair, leaves simple, densely covering the twigs spreading in all directions, each leaf 1.5 -2.3 cm long; the cones are bluish in colour, seed are winged. Leaves flat, 1 to 5.5 cm long, about 2 mm broad; shining, midrib in the upper surface channelled down the middle but raised beneath; with two faint white lines on either side of the midrib beneath, petiole very short, colour- greyish-brown; odour- terebinthinate like; taste astringent.

Part used: Leaves
Chemical constituents: Petroleum ether extract was a dark green color & presented phytoconstituents; lipids, flavonoids, triterpenoids, & steroids. The chloroform extract was brownish-red color & presented phytoconstituents; alkaloids & flavonoids. Ethyl acetate extract was reddish color & presented phytoconstituents; tannins, amino acid flavonoids, triterpenoids, & steroids. The methanol extract was reddish-brown color & presented phytoconstituents; saponins, alkaloids, amino acids, flavonoids, triterpenoids, & steroids. There was found that a new alkaloid namely 1-(4'-methoxyphenyl)-aziridine from the leaf of A. webbiana& its chemical structure was elucidated based on elemental & spectral analysis. has been isolated, a new biflavonoid, Abiesin from the leaves of A.webbiana& identified as 5,3″,7″- trihydroxy- 7,4′,4‴-trimethoxy-(3′,6″)-biflavone [43-44].

Pharmacological evaluation of drugs of 'arathai kudineer churnam'
Immunomodulatory activity
Alpinia galanga
Bendjeddou, D. et al (2003) reported the Immunostimulating activity of the hot water-soluble polysaccharide extracts of Alpinia galanga in mice. *Alpinia galanga* showed a marked stimulating effect on the reticuloendothelial system (RES) & increased the number of peritoneal exudate cells (PEC), & spleen cells in mice [47] Jain Alok et al., studied

Immunomodulatory & anti-oxidant antioxidant effects of different extracts of Alpinia galanga Linn. in a dose-dependent manner. The immunomodulatory activity was studied by examining T cell proliferation, & splenocyte proliferation, & by delayed-type hypersensitivity reaction measurement. The antioxidant effect was observed by measuring lipid peroxidation (LPO), reduced glutathione (GSH) content, & determination of superoxide dismutase (SOD) & catalase (CAT) activities. The study concluded that the flavonoid fraction of Alpinia galanga Linn. rhizomes has greater immunostimulating effects as well as antioxidant effects in mice [48].

Glycyrrhiza glabra

In vitro studies proved that G.glabra at 100μg/ml concentration possesses immunostimulatory effects. It increases the production of TCD69 lymphocytes & macrophages from human granulocytes. Licorice root extract was found to prevent the rise in the number of immune complexes related to autoimmune diseases like systemic lupus erythematosus [49]. P M Mazumdar et al., studied the immunomodulatory properties of roots of *Glycyrrhiza glabra (G. glabra) &*significant potentiation in immunomodulation occurs or not with the combination of zinc. Immunomodulatory potential of aqueous liquorice extract (ALE) 0.75 & 1.5 g/kg BW & its combination with zinc (45 mg/kg BW) were determined by the effect on leukocyte count & spleen weight, *In vivo* phagocytosis (carbon clearance method), determination of cellular immune response, haemagglutination antibody titre & plaque forming cell (PFC) assay using sheep red blood cells (SRBC). The present study has shown the immunomodulatory activity of aqueous root extract of *G. glabra* L. at the dose 1.5 g/kg BW. *G. glabra* L. in combination with zinc has shown potentiation of immunomodulatory activity in all aspects of the study [50]. Bagherwaletal.,evaluated the Aqueous extract of roots of *Glycyrrhiza glabra* for immunomodulatory activity using *E.coli* induced abdominal sepsis.The results of the study was compared with the control group. The extract was found to be effective in reducing mortality in *E. coli* induced abdominal sepsis. The immunostimulant effect was observed with increased phagocytosis in carbon clearance test [51]. Hussain K et al., evaluated the immunomodulatory activity of aqueous methanolic extract (AME) of Glycyrrhiza glabra (roots) against mixed Eimeria species infection in broiler chickens. Cell mediated immune response was evaluated by four tests (Phytohemagglutinin-P, Concanavalin-A,Carbon clearance assay & Dinitrochlorobenzene). Humoral immune response was evaluated by microplate hemagglutination test using sheep red blood cells. Results revealed a dose dependent immune response in G. glabra AME treated groups [52].

Piper longum

Mananvalan & Singh reported the immunomodulatory potential of P. longum fruits extract has been evaluated by hemagglutination titre, macrophage migration index, & phagocytic index in mice. A well-known ayurvedic preparation containing long pepper *pippalirasayana*, was tested in mice infected with *Giardia lamblia* & found to produce significant activation of macrophages as shown by an increased macrophage migration index & phagocytic activity [53]. The immunoregulatory potential of P. longum &piperinic acid, one of its active constituents, in Balb/C mice (in vivo) & human PBMCs (in vitro) models showed a dose dependent decrease of lymphocytes (CD4+ & CD8+ T cells) & cytokine levels in sensitized Balb/C mice with a marked inhibition [54]. Alcoholic extract of the fruits of P. longum & its component piperine was studied for their immunomodulatory & antitumor activity. Alcoholic extract of the fruits &piperine were found to be cytotoxic [55].

Alpinia officinarum

P S Ediriweera et al. studied the immunostimulative effect of Alpinia calcarata extract combination on cyclophosphamide-induced immunosupression in rats. Differential white blood cell (WBC) count, leukocyte adhesion, IL-4, IL-10, & IL-12 levels were measured in all rats after the treatments. Subsequently, animals in each group were orally fed with 1 ml of cyclophosphamide solution at a concentration of 20 mg/ml. The study concluded that the ethanolic extract combination of the plant species exerts its immunomodulatory effect via cytokine expression & can attenuate the immunosuppression induced by cyclophosphamide [56].

Abbies webbiana

Ryang et al. studied the single component paclitaxel obtained from fermentation broth by molecular imprinting technique, & its antiviral, antitumor, & immunomodulatory activities. The inhibition of HIV-1 protease activity was concentration-dependent. Paclitaxel significantly up-regulated the expression of interleukin-6 [57].

CONCLUSION

There is a constant increase in the number of cases & the potential possibility of the health care system across the country getting overwhelmed, it is amply clear that the effective way of approach to protecting the unaffected population should be aimed at boosting the immunity. This is where the potential role of the traditional system like *Siddha* needs to be harnessed. There is a greater emphasis now on improving immunity through diet, lifestyle, & regimen involving immense utility in the science of Siddha. This literature analysis was performed to have a scientific perspective on this *Siddha* polyherbalformulation '*Arathai kudineer churnam*' which highlighted pharmacognostic, Siddha concept of herbal properties along with an emphasis on the immunomodulator, Anti-viral, Anti-oxidant, Anti-inflammatory activities of herbs used in this formulation. Based on the above facts it is pertinent to note that, certain bioactive compounds present in the herbs exhibited inhibitory activity against SARS-CoV2 in molecular docking. Studies also proved its Anti-oxidant, Anti-inflammatory, & Immunomodulatory action which can stimulate both the cellular & the humoral immune responses suggesting its therapeutic usefulness. So, the effects of various phytochemicals present in '*Arathai kudineer churnam'* will help in suppressing & curing the clinical symptoms associated with COVID-19. Hence, a more detailed investigation has to be carried out for obtaining a scientific approach to this formulation & developing it as a viable therapeutic alternative against COVID-19 infection.

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Siddha Concepts About Constituents of *Arathai kudineer churnam*:[45-46]

Table I :describes the ingredients of *Arathai kudineer churnam* with the taste of each drug, Analysis of humoral theory, its five-element perspective, parts used, & actions of the drug.

S. no	Name of the drug	Common name	Suvai (Taste)	Pancha Bootham	Action on humour	Parts used	Actions
1	*Alpinia galanga (L.) Willd. (Perarathai)*	Greater galangal	Karpu (Acrid)	Air + Fire	Pacifies vitiated ***Kabam***	Rhizome	Respiratory ailments Febrifuge
2	*Glycyrrhiza glabra (Athimadhuram)*	Liquorice	Sweet (Inippu)	Earth+Water	Pacifies vitiated ***Pitha*** &***Vatha***humours	Root	Tonic Emollient, Laxative Mild expectorant
3	*Piper longum L. (Thippili)*	Long pepper	Ripened-karpu (Acrid)	Air + Fire	Pacifies vitiated ***Kabam***	Fruit	Stimulant Carminative Stomachic

4	*Alpinia officinarum Hance (Citrarathai)*	Lesser galangal	Karpu (Acrid)	Air + Fire	Pacifies vitiated ***Kabam***	Rhizome	Expectorant Febrifuge Stomachic
5	*Abies webbiana (Wall. ex D. Don) Lindl (Thalisapathiri)*	Indian silver fir	Karpu (Acrid)	Air + Fire	Pacifies vitiated ***Kabam***	Leaves	Stomachic Carminative Expectorant Tonic Bronchodilator

Table 2: Scientifically proven antiviral activity of constituents of AKC with special mention toCOVID -19

S. No.	Botanical name	Antiviral studies
1	*Alpinia galanga*	Phenylpropanoid in galangal shows potential inhibitor to SARS-CoV2 against 3 target proteins, RBD-S, PD-ACE2, & SARS-CoV-2 protease in molecular docking studies [58].19S-19- Acetoxychavicol acetate inhibits the replication of Human immune deficiency virus by blocking the transport of the Regulatory HIV-I protein (Rev)& against human cytomegalovirus (HCMV) [59] *In vitro* cytotoxic assay of aqueous ethanolic extract of A. Galanga against Chikungunya virus Using Vero cell lines [60].
2	*Glycyrrhiza glabra*	Glycyrrhizin exhibits antiviral activity against SARS Cov-2, respiratory syncytial virus, arboviruses, vaccinia virus, herpes simplex type 1, Newcastle disease, & vesicular stomatitis viruses in vitro. anti-HIV against MT-4 cells, dengue, Japanese encephalitis, yellow fever, mammalian tick-borne encephalitis, influenza, & hepatitis A, B, & C viruses [61-64]
3	*Piper longum*	Methanolic /chloroform extract of Piper longum against Vesicular stomatitis virus & human para influenza virus on HeLa cell lines [65]. Piperine shows remarkable inhibitory HBV activity against hepatitis B [66].
4	*Alpinia officinarum*	Anti-viral against diarylheptanoids isolated from Alpinia officinarum against the respiratory syncytial virus, poliovirus, measles virus, & herpes simplex virus type 1 in vitro [67]. Compounds in A. officinarum are Potent SARS-CoV-2 Papain-like Protease Inhibitors [68]. *In vitro cytotoxic* assay of aqueous ethanolic extract of *Alpinia Officinarum* Hance against Chikungunya virus Using Vero cell lines. Anti-influenza virus activity Diarylheptanoids & AO-0011 from Alpinia officinarum exhibit anti-influenza virus effect *in vitro* [69].
5	*Abbies webbiana*	Taxol, Baccatin III, exhibits strong interactions with the targets of SARSCoV2 & human ACE2 in molecular docking[70]. paclitaxel has inhibitory activity on HIV-1 virus [71], human breast cancer (MCF-7 cell),& inhibitory action on HSV-1 virus in Vero cells [72].

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RESEARCH ARTICLE

Evaluation of Polyherbal Mixtures against Glycerol Induced Diabetic Nephropathy in *Albino wistar* Rats

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ABSTRACT

This study evaluates the polyherbal mixtures against Glycerol induced Diabetic nephropathy in *Albino wistar* rats. The present study was to investigate the effect of polyherbal mixtures against Glycerol induced Diabetic nephropathy in *Albino wistar* rats. The polyherbal formulation was prepared by mixing Three integrated extracts of *Aegle marmelos*, *Ocimum sanctum* and *Syzygium cumini*. The extracts were obtained by Cold maceration process. Diabetes mellitus was induced in wistar rats by the administration of Streptozotocin at a dose of 65mg/kg (i.p.) injected, after 15min Nicotinamide was administered at a dose of 110mg/kg (i.p.). After that Diabetic Nephropathy was induced by administrating the Glycerol at a dose of 10ml/kg by intramuscular injection into both the hind limbs. The total treatment period was about 18 days. The Diabetic rats were treated with Pioglitazone (10mg/kg orally) and a polyherbal formulation at two dose levels i.e. (200 and 400 mg/kg by oral route). Extract was given orally 60 min before to Glycerol injection. Animals administered PHF along with Glycerol in diabetics experienced a significant reduction in Fasting blood sugars & Serum biochemical parameters such as BUN, creatine, urea, uric acid & increase in Total protein, Total albumin & also urine output compared to Disease control groups. This finding suggests that the treatment with PHF showed significant nephron-protective effect against STZ-NAD induced DN.

Keywords: Diabetic Nephropathy, Streptozotocin, Nicotinamide, Pioglitazone, Polyherbal extract and Renal function test.

INTRODUCTION

Diabetes mellitus is a disease, in which homeostasis of carbohydrates, proteins, and lipid metabolism is improperly regulated by hormone insulin resulting in the elevation of fasting blood glucose levels [1]. and also characterized by hyperglycaemia along with long-term complications which effects on kidneys, eyes, nerves and blood vessels named as diabetic nephropathy, diabetic retinopathy, diabetic neuropathy and atherosclerotic coronary artery disease. Diabetic nephropathy (DN) is one of the most serious complications in diabetes mellitus and also most common source of end-stage renal disease (ESRD) [2]. ESRD due to diabetes has been estimated to be 30-47% of all incident cases worldwide [3]. Previous reports also suggest that 43% of the chronic renal disease (CRD) patients on dialysis have DN, 60% death cases of diabetes mellitus patients are due to DN, and death cases of diabetes mellitus patients due to renal failure are 17 times more as compared to non-diabetes mellitus patients [3]. A typical morphological change in the diabetic kidney involves an increase in kidney size and weight, increase glomerular volume, accumulation of extracellular matrix in glomeruli that correlates with the loss of renal function such as mesangial expansion, tubulointerstitial fibrosis, and irreversible deterioration[5,6]. DN is a pathological progression from hyper-filtration to micro-albuminuria then to macro-albuminuria and finally to renal failure [7]. A number of factors are important for the development of DN, including hyperglycemia, hypertension, oxidative stress, and inflammation, and have been shown to lead to histological changes [8]. Angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor antagonists are the main therapeutic agents presently seem to produce partial reduction in proteinuria and attenuate progression of CRD to ESRD. However, many patients do not respond to these agents and their progress to ESRD at an early stage. The concept of polyherbals have been emphasized in Sharangdhar Samhita, an Ayurvedic literature dating back to 1300 AD. When mixing the multiple herbs in a same ratio, it gives a better therapeutic effect and reduce the toxicity [9]. PHF increases the therapeutic action and reduces the concentration of single herbs so that it reducing adverse events. In the recent studies, herbal formulations have gained greater importance than ever before, mainly due to their efficacy and easy availability as well as less side effects as compared to the synthetic drugs [10].

World Health Organization (WHO) also estimated that 80% of the world's people still rely mainly on traditional medicines for their health care. *Aegle marmelos, Ocimum sanctum* and *Syzygium cumini* are well-known herbs available throughout India and they are commonly used for the treatment of various diseases including diabetes mellitus and also various diabetic complications. All the extracts of herbs used for the study were obtained by Maceration process and these extracts are called Integrated extracts. They are pure, highly powerful and extremely concentrated and are free from any residues of chemical insecticides, pesticides & herbicides [11]. The main objective of the present study is to prepare the polyherbal formulation of Integrated extracts and evaluate its nephro-protective activity in the STZ-NAD induced diabetic nephropathy in rats and to check PHF treatment could have a synergistic effect with pioglitazone. Pioglitazone, which acts on reversing almost all mechanisms involved in the pathogenesis, is the new recommended preventive option in this study [12,13]. To the best of our knowledge, no investigations have been made on this prevention, although several previous studies have designated its Reno protective effects in other nephropathy models [14,16]. Pioglitazone is an oral antidiabetic agent and is classified as a peroxisome proliferator activated receptor-γ (PPAR-γ) agonist (a member of thiazolidinediones), which binds to a specific site on the DNA helix. It controls the transcription of numerous target genes and participates in the regulation of several vital processes such as adipocyte differentiation and lipid and carbohydrate metabolism [11,12].

Objective

The Objectives were to evaluate the nephroprotective activity of ethanolic extracts of *Aegle marmelos, Ocimum sanctum, Syzygium cumini* leaves in Glycerol-induced Diabetic nephropathy in *Albino wistar* rats and to find out the Probable mechanism.

MATERIALS AND METHODS

The study was initiated after getting approval of IAEC (Institutional Animal Ethics Committee), Vignan institute of pharmaceutical technology. (Reg. no. 2003/PO/RE/S/18/CPCSEA).

Drugs and Chemicals

Streptozotocin, Nicotinamide, Glycerol and Pioglitazone was procured from Sai chemicals, Visakhapatnam. All other chemicals which are used in this study were of Analytical grade.

Procurement of Plants

The selected plants of *Aegle marmelos, Ocimum sanctum and syzigium cumini* are collected from the local region of Visakhapatnam. The plant parts like Leaves and twigs of *Ocimum sanctum* and leaves of *Aegle marmelos* and *Syzygium cumini* are subjected to shed dried and powder under Cold maceration process.

Extraction procedure

The three plants of leaves and twigs were washed with distilled water and subjected to shade dried for 15 days with proper protection from the dust particles. Then the dried leaves and twigs are powdered mechanically by the help of grinder and poured into sieve to obtaining the coarse powder. After that the three plant powders are transferred into separate Beakers then add 70% of Ethanol & 30% water as a solvent individually then the solvent mixtures cover with an Aluminium foil & kept aside then stirred thoroughly for 1 week to get the Ethanolic extract. The solvent mixtures are filtered using muslin cloth. The filtered solvent is poured into a Round bottomed flask to undergoing distillation process. After the completion of distillation process semisolid extracts were obtained then the semisolids extracts were kept in Desiccator to evaporate the moisture content.

Dose Selection

The dose for the Preparation of Polyherbal mixture is selected from the Literature review. The Plant extracts of *Aegle marmelos, Ocimum sanctum and Syzygium cumini* have Anti- Diabetic, Anti-inflammatory, Anti- Microbial & Anti-Oxidant activity. The dose of these selected Polyherbal extracts were predicted by Acute Toxicity study.

Acute toxicity studies

Acute toxicity study was performed to determine dose according to OECD guidelines 423[17]. The rats showed no mortality at a dose of 2000mg/kg, during time period of 14 days. Hence, two dose levels were selected, in which the first dose was one-eighth of the upper dose and the other was twice that of the one-eighth dose (200 and 400 mg/kg) selected for *in vivo* studies. Therefore, the dose of polyherbal integrated extracts were selected as an intermediate dose for the preparation of polyherbal mixture.

Experimental study

Animals

The Healthy male Albino wistar rats weighing about 200-250g were procured from albino research lab., Hyderabad, A.P, India. The animals were well housed under standard well maintained 12: 12 dark and light cycle in standard environment (temperature 23 ± 1° C) with relative humidity 50-60%. The animals were free access to water and ad libitum with standard diet. The present study was approved by the institutional animal ethical committee (IAEC) bearing registration IAEC/ VIPT/ 2021/05.

Induction of DM:

DM was induced in overnight fasted adult *Albino wistar* rats by a single intraperitoneal injection of 65 mg/kg streptozotocin (STZ), after 15min Nicotinamide (NAD) was administered at a dose of 110mg/kg in i.p. route. STZ was dissolved in citrate buffer (pH 4.5) and NAD was dissolved in normal saline. Blood glucose levels were

measured from tail vein using glucose meter, four days after induction diabetes. Rats with blood glucose levels above 150mg/dl, were considered as diabetic and were used for further study by initiating the treatment.

Induction of glycerol-induced

Diabetic Nephropathy was induced by intramuscular administration of a single dose of 50% hypertonic glycerol (10 ml/kg), The required amount of glycerol was administered as a deep i.m. injection equally distributed into both the hind limbs. Thirty Healthy male *Albino wistar* rats, weighing 200-250g were used in the present study. They were divided randomly into six equal groups of 5 animals each. The animals were permitted free access to food, but deprived of drinking water for 24 h before glycerol injection.

Grouping of animals and treatment protocol

All the 30 animals were divided into 5 groups, each Group having 6 animals (n=6). The wistar rats had free access to food but were deprived from drinking water 24h before glycerol injection [18]. Group1 was treated with normal saline (10 ml/kg) per oral, group 2 with STZ-NAD along with a single dose of hypertonic glycerol (10 ml/kg) by intramuscular injection into both the hind limbs induced diabetic group, group 3 STZ-NAD with glycerol and also treated with Standard pioglitazone at a dose of 10mg/kg administered orally, group 4 STZ-NAD along with glycerol and treated with PHF-A at a dose of 200 mg/ kg and group 5 was STZ-NAD along with glycerol and also treated with PHF-B at a dose of 400mg/kg. The PHF extract was given by oral route 60 min prior to glycerol injection. The animals were observed for general behaviour and activity. All the groups received the above treatment for about 14days.

Biochemical Parameters

Serum was collected on respective days is used for the estimation of BUN, Creatinine, urea, uric acid, Total protein and Total albumin levels. Urine output was measured for all the animals for 24 h.

Statistical Analysis

Statistical analysis was performed by using Graph Pad prism in statistical software. The values were expressed as mean ± SEM and analysis were done by one-way analysis of variance (ANOVA) Followed by Dunnet's test for multiple comparisions and statistical significance was set at $p<0.001$.

DISCUSSION

Diabetic nephropathy is one of the most serious micro-vascular complications of both type 1 and type 2 diabetes and it is one of the leading causes of end-stage renal disease in type 2 diabetes. The pathogenesis of diabetic nephropathy is multifactorial, though chronic hyperglycaemia plays a crucial role [19]. The present study has to evaluate the effects of Polyherbal extracts of STZ- NAD of Glycerol induced acute renal failure in male Albino wistar rats. Administration of STZ-NAD in albino wistar rats produced and sustained increase in blood glucose level. STZ causes diabetes by the rapid depletion of β-cells of pancreas. Moreover, when administration along with NAD, it causes minor damage to pancreatic β-cells [20], it results in elevation of blood glucose levels. Expression of elevated fasting blood glucose levels confirmed the induction of diabetes in Streptozotocin induced experimental rats [21]. Glycerol induced acute renal failure is one of the most common animal models used for evaluation of Nephro-protective activity [22,23]. Increased levels of Serum creatinine, Serum urea, uric acid and BUN are the markers of DN. Diabetes induced in rats were associated with the characteristic loss of body weight, which is caused due to increased muscle wasting and loss of tissue proteins [24,25]. In our study Polyherbal integrated extracts has shown protective effect evidenced by an inhibition of an alteration of renal biochemical parameters and reversal of histopathological changes. The significantly increased levels of BUN, Serum creatinine, serum urea, serum uric acid and significantly decrease levels of serum total protein and serum albumin levels in Glycerol induced DN in rats. In the present study the administration of polyherbal integrated extracts has showed significant reduction in Serum creatinine, Blood urea Nitrogen, Serum urea, Uric acid and increase in total proteins and albumin as compared to

diabetic control rats. In addition, the urine output is increased in Polyherbal treated animals. Treatment of diabetic rats with PHF reduces renal oxidative stress, it indicating the antioxidant potential of polyherbal formulation[26]. However, co-administration of PHF and pioglitazone has a more beneficial effect than when administered singly. The phytochemicals found to be present in the polyherbal integrated extracts have Flavonoids, terpenoids, alkaloids, tannins and saponins [27,28]. Among these flavonoids could be responsible for the antioxidant's property as these phytoconstituents are already reported to have antioxidant activity. Thus, the invitro and in vivo findings suggest that protection from antioxidant activity from various phytochemicals present in polyherbal integrated extracts could have nephroprotective actions of polyherbal extracts in Diabetic nephropathy. During this study, no noticeable responses were seen in rats. This helps to predict that it does not contain any type of toxicity and is safe. In this study Standard drug pioglitazone (10mg/kg) & Polyherbal formulation extracts showed significant activity at a dose level of 200 and 400mg/kg, whereas Polyherbal preparation at a dose of 400mg/kg is found to have significant nephroprotective action.

CONCLUSION

The present study confirms that the selected plants extracts and polyherbal mixtures were shown to have beneficial role in control of blood glucose levels as well as in the prevention the development of complication such as DN.The Ocimum sanctum, Syzygium cumini, Aegle marmelos are responsible for nephroprotective action. The various active constituents of selected plant leaves have flavonoids, tannins, saponins, coumarins, alkaloids terpenoids. Among these, flavonoids could be responsible for antioxidant property as these phytochemical constituents are already reported to have antioxidant property. Std (pioglitazone 10mg/kg), PHM A (200mg/kg) and PHM B (400mg/kg) treated rats showed significant decrease in the FBS, BUN, serum creatinine, serum urea, serum uric acid (P***< 0.001) whereas, increased in total protein, total albumin and urine output compared with diseased rats done by DUNNETT'S multiple comparison test. This finding suggests that the treatment with polyherbal mixtures (PHM B> PHM A) showed significant nephroprotective effect. Since, the above polyherbal mixtures have yielded better results when treated on animals, they can also be studied on humans for the development of newer drugs for the betterment of life standards of diabetic patients. Since the above polyherbal formulation extracts have yielded better results when treated on animals, they can also be studied on human trials for further development of newer drugs for the betterment of life standards of diabetic patients.

ILLUSTRATIONS

The estimated values of biochemical parameters in all the experimental rats during the treatment period are summarised in table 2 and Figures. All Values are expressed as (MEAN ± SEM) of fasting blood sugar, BUN, urea, creatinine, uric acid, total protein, total albumin in serum and urine output. Stastical analysis is done by One way ANOVA followed by Dunnett's multiple comparision test and found significantly different at P value is found to be **p<0.01 when comparing with disease control and ## p<0.001 when diseased control is compared with normal control.

Conflict of interest: We have no conflict of interest regarding this research work.

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Table 1: Grouping of animals and treatment protocol

GROUPS	TREATMENT
GROUP-1 Normal control	Vehicle i.e., Normal Saline (10ml/kg) by Orally.
GROUP-2 Diabetic Control	Streptozotocin- Nicotinamide
GROUP-3 Standard	Streptozotocin- Nicotinamide, 50% Glycerol (10ml/kg) & Pioglitazone (10mg/kg)
GROUP-4 PHM A (200mg/kg)	Streptozotocin- Nicotinamide, PHM-A, I.M injection of 50 % glycerol (10ml/kg) equally divided into both hind limbs
GROUP-5 PHM B (400mg/kg	Streptozotocin- Nicotinamide, PHM-B, I.M injection of 50 % glycerol (10ml/kg) equally divided into both hind limbs

Table 2: Effect of Polyherbal formulation extracts on Fasting blood sugars, Serum biochemical parameters & urine output

GROUPS	Fasting blood sugar		Serum BUN	Serum Creatinine	Serum Urea	Serum Uric Acid	Total Protein	Total Albumin	Urine output
	0th Day	14th Day							
NC	86.33 ± 2.5	84.83± 3.927	15.67± 0.126	0.585± 0.020	9.873± 0.121	0.648± 0.038	5.606± 0.078	3.83± 0.035	7.246± 0.060
Glycerol +DC	#216.5 ± 0.8	#375.5± 6.360	#24.11± 0.115	#1.323± 0.050	#29.13± 0.213	#1.563± 0.025	#2.191± 0.029	#2.256± 0.043	#3.521± 0.046
Glycerol +Pioglitazone	214.33 ± 1.3	102.6± 5.535	16.20± 0.134	0.698± 0.033	12.63± 0.159	0.686± 0.041	5.661± 0.090	4.256± 0.043	8.501± 0.088

Glycerol +PHM A (200mg/kg)	218.33± 0.7	163.5± 8.574	19.66± 0.173	0.913± 0.022	19.76± 0.093	0.95± 0.058	7.085± 0.090	4.801± 0.033	7.326± 0.083
Glycerol +PHM B (400mg/kg)	*218 ± 0.5	*110.83± 7.880	*17.89± 0.074	*0.808± 0.052	*14.66± 0.098	*0.768± 0.034	*6.206± 0.079	*4.511± 0.044	*7.651± 0.085

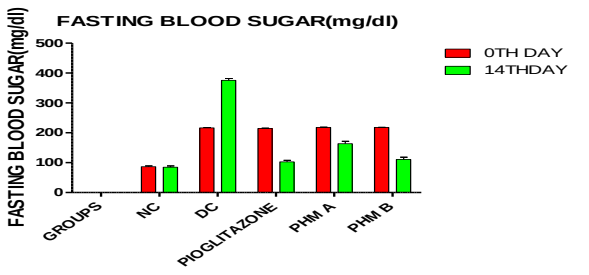

Figure 1. Effect of PHM on Fasting blood sugar levels in different groups

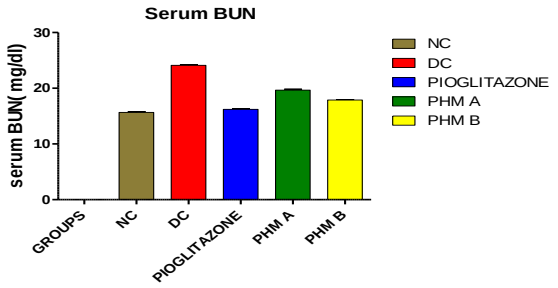

Figure 2. Effect of PHM on Serum BUN levels in different groups of rats.

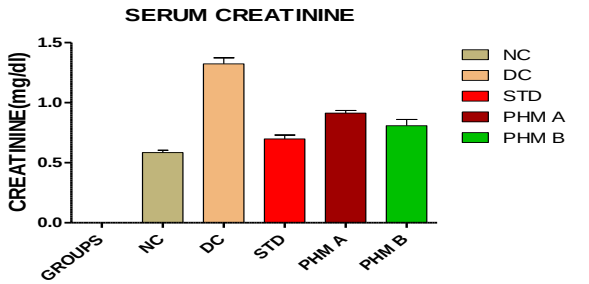

Figure 3. Effect of PHM on Serum Creatinine levels in different groups of rats.

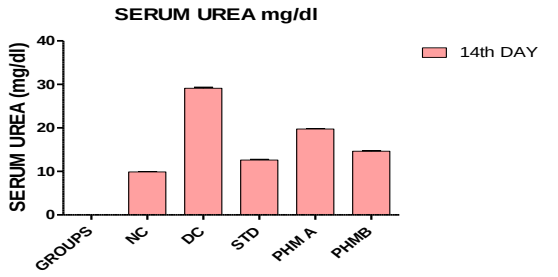

Figure 4. Effect of PHM on Serum Urea levels in different groups of rats.

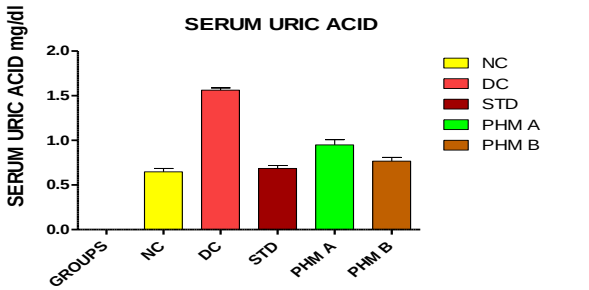

Figure 5. Effect of PHM on Serum Uric acid levels in different groups of rats.

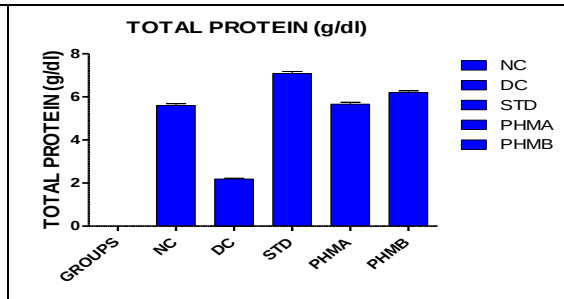

Figure 6. Effect of PHM on Total protein levels in different groups of rats.

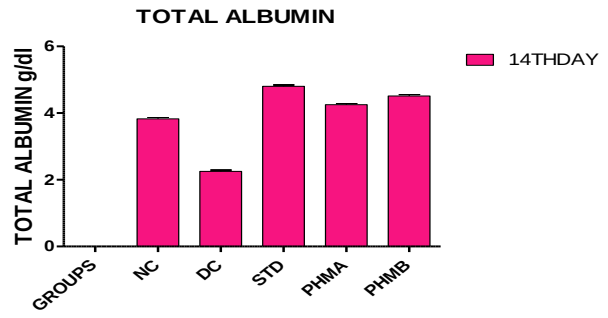

Figure 7. Effect of PHM on Total Albumin levels in different groups of rats.

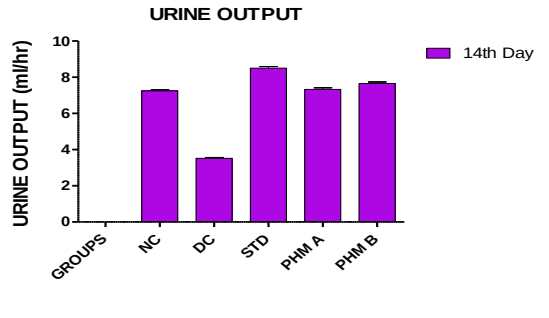

Figure 8. Effect of PHM on Urine output levels in different groups of rats.

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RESEARCH ARTICLE

Effect of Zinc Fertilization on Maize Yield and Yield Components

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ABSTRACT

The deficiency of macro and micro nutrients limits the growth and development capacity of major crops particularly, the executive crops such as corn. The impact of zinc treatment on maize productivity should be quantified. A field experiment was conducted at farmer field, during summer 2022.We experimented with the various remedies *Viz*., T_1-Control (RDF), T_2- Seed priming @ 1% $ZnSO_4$,T_3-Foliar application of 1% $ZnSO_4$, T_4-Soil application of $ZnSO_4$ @ 25 kg ha^{-1}, T_5-Soil application of $ZnSO_4$ @ 37.5 kg ha^{-1} , T_6- Seed priming @ 1% $ZnSO_4$ with foliar application of 1% $ZnSO_4$, T_7-Seed priming @ 1% $ZnSO_4$ with soil application of @ 25 kg ha^{-1}, T_8-Seed priming @ 1% $ZnSO_4$ with soil application of @37.5 kg ha^{-1},T_9- Seed priming @ 1% $ZnSO_4$ with foliar application of 1% $ZnSO_4$ at 40 and 60 DAS Plus soil application of $ZnSO_4$ @ 25kg ha^{-1},T_{10}- Seed priming @ 1% $ZnSO_4$ with foliar application of 1% $ZnSO_4$ at 40 and 60 DAS Plus soil application of $ZnSO_4$ @ 37.5kg ha^{-1}.The seed priming with 1% $ZnSO_4$ plus soil application of $ZnSO_4$ @ 25 kg ha^{-1} had resulted in Greater leaf area (529.7 cm), Thousand grains weight (346.9 g), Grains cob^{-1}(596), Biological yield (12627 kg ha^{-1}) and Grain yield (4898 kg ha^{-1}).As a result, it was determined that using seed priming 1% zinc solution with soil applied zinc at a rate of 25 kg ha^{-1} had boosted maize growth, biological yield, grain yield and yield contributing characteristics.

Keywords: Maize, Zinc, Growth and Yield.

INTRODUCTION

One of the innovative methods that has a good impact on plant growth, yield and metabolism is seed priming. Pre-sowing seed treatment range from hydropriming, chemical and biological to physical ones (Farooq et al., 2020). Seed priming with micronutrients has of late gained momentum in various research programs (Kumar et al., 2020). Nutrient seed priming is widely used in parts of Asia with studies in Bangladesh, Nepal, India and Pakistan showing

the NSP can increase maize yields by up to 70% after adding such nutrients as molybdenum, zinc, boron and phosphate to priming water (Harris et al., 2001). Harris et al.(2007) showed positive responses of chickpea and wheat to priming with $ZnSO_4$ in moderately Zn-deficient soils. Halopriming is more commonly used than other strategies since it is relatively simple for farmers to handle and has been shown to increase seed performance in the field in terms of better germination rate, metabolic pool and stress tolerance. Maize (*Zea mays* L.) belongs to family poaceae and is the third most important cereal crop after wheat and rice in India. It has more nutritional value and contains about 72% starch, 10% proteins, 4.8% oil, 8.5% Fiber,3 % sugar and 1.7% ash (Chaudhary, 1993). A micronutrient with a variety of roles in plant physiology is zinc. It participates in the control of various primary and secondary metabolic enzymes with a focus on photosynthesis and hormone regulation. For years, plant scientists and farmers have been working on zinc supplementation. Major emphasis has been upon addition of zinc salts in rooting medium (Dwivedi et al., 2020). Root supplementation requires a lot of chemicals, which makes it expensive and unfriendly to the environment. Zinc (Zn) is an essential micronutrient and its deficiency is the main cause of reduced shoot growth. In maize, higher than adequate shoot Zn concentrations reduces the shoot growth suggesting the P toxicity but induces the Zn deficiency also thus P application rates should be chosen carefully. Nutrient seed priming is thus a cheaper way of adding micronutrients compared to expensive methods like foliar spraying. Other benefits of seed priming include increased seedling tolerance to stress. It was hypothesized that the seed priming with zinc sulphate could modulate primary and secondary metabolism of maize and hence improves growth and yield potential.

MATERIALS AND METHODS

A filed experiment with selected dose ($ZnSO_4$) was planned with high analytical grade, purity(>99 %) as seed priming agents. Maize seeds were subjected for presowing seed treatment with zinc sulphate (Hydroprimed 1% solution) from 12 hours. The seeds were completely dipped in the solutions. To find out the impact of zinc on the maize production a field experiment was conducted at farmer holding land during summer 2022. The experiment was carried out in randomised block design with three replication and Ten treatments *Viz*., T_1-Control (RDF), T_2-Seed priming @ 1% $ZnSO_4$,T_3-Foliar application of 1% $ZnSO_4$, T_4-Soil application of $ZnSO_4$ @ 25 kg ha^{-1}, T_5-Soil application of $ZnSO_4$ @ 37.5 kg ha^{-1} , T_6- Seed priming @ 1% $ZnSO_4$ with foliar application of 1% $ZnSO_4$, T_7-Seed priming @ 1% $ZnSO_4$ with soil application of @ 25 kg ha^{-1}, T_8-Seed priming @ 1% $ZnSO_4$ with soil application of @37.5 kg ha^{-1},T_9- Seed priming @ 1% $ZnSO_4$ with foliar application of 1% $ZnSO_4$ at 40 and 60 DAS Plus soil application of $ZnSO_4$ @ 25kg ha^{-1},T_{10}- Seed priming @ 1% $ZnSO_4$ with foliar application of 1% $ZnSO_4$ at 40 and 60 DAS Plus soil application of $ZnSO_4$ @ 37.5kg ha^{-1}.The net plot size was 4.5×5.0 M, having (24.25 M^2) having 6 rows 75 cm apart. The nitrogen, phosphorus and potassium were applied as per the recommended dose. When P& K were applied full dose in basal, where as nitrogen 50% only applied at basal while remaining amount of urea was applied as per the stage(DAS) i.e. Two split dose. For foliar application of zinc 1% solution of $ZnSO_4$ .H_2O was prepared. The solution was diluted with water. In addition as per the treatment soil application of zinc sulphate recommended. Maize cultivar CO 6,used as a test crop . first irrigation was given 7 days (One week) after sowing, while the subsequent irrigation was adjusted according to the need of the crop and avoiding the over irrigation strictly. The agronomic practices such as irrigation, hoeing and thinning were kept normal and uniform for all the treatments. The biometric observation such as leaf area, cobs M^{-2}, No. of plants ha^{-1}, thousand grains weight, grain cob^{-1}, biological yield and grain yield was recorded.

Leaf area (cm^2)

The seed priming, soil and foliar application of zinc significantly increased the leaf area in maize over control(Table 1). The greater leaf area(529.7 cm^2) was recorded when applied seed priming of 1% zinc solution with soil application of $ZnSo_4$ @ 25kgha^{-1} compared to the control plots(342.5 cm^2), which is about 35% higher than control treatments. Zn exerts a greater influence on basic plant life processes such as nitrogen metabolism, uptake of nitrogen and photosynthetic activities. These results are in accordance with the finding of Yordanov et al., (2003) who reported that the leaf area index (LAI) was increased in maize by priming of seeds with 1.5% $ZnSo_4$ solution. Greater leaf area

index values may attribute to significant increase in leaf expansion due to good germination and growth of plants as affected by priming.

Yield Attributes

Number of grain cob^{-1} and thousand weight(g) at seed priming with 1% $ZnSo_4$ solution and soil applied $ZnSo_4$ @ 25kgha^{-1} higher number of grains weight(346.9 g) was obtain as compare to control plots(Table). The higher grains cob^{-1} and 1000 grain weight in Zn applied plots could be attributed to the microelements effects on leaf area development, greater assimilate production. In addition more activity of enzymes involved in sucrose metabolism that caused increase in test grain weight. Similar results were reported by Harris et al. (2002) and Bakht et al.(2010) who reported that primed seeds produced larger grains than un-primed seeds. The current results are supported by the findings of Mauromicale et al.(2000) who reported early flowering, maturity time and yield of a crop due to early seedling growth and enhanced plant nutition because of priming. These findings also in line with Yousaf et al.(2011) who observed that priming treatment significantly improved yield attributes of crops.

Yield (kgha^{-1})

Biological and grain yield (12627 and 4898 kgha^{-1}) was recorded when seed priming with 1% $ZnSo_4$ solution and soil application of $ZnSo_4$ @ 25kgha^{-1} was applied as compared to control plots presented in table .This increased could be associated with the grounds that seed priming with micronutrients increased seed yield and this increase can be related to rapid, optimal establishment of the plants and their greater ability in using nutrient. The primed seed emerge first and more uniform and seedling grown more vigorously, leading to a wide range of phonological and yield related benefits. Therefore, better use of nutritional resources due to early emergence of plants on eventually result in higher seed yield of cereleals crops (Badiri et al., 2014). Higher yield of maize due to Zn priming is attributed to the enhanced synthesis of carbohydrates and their transport to the site of grain production (Pedda babu et al.,2007).

CONCLUSION

In nutshell, it may be concluded that seed priming of maize seeds with 1% $ZnSo_4$ and soil application of $ZnSo_4$ @ 25kgha^{-1} may be useful for proper germination, better crop establishment and enhanced yields.

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Table-1 Effect of seed priming with zinc sulphate on yield components

Treatment details	Leaf area(CM^2)	Cobs M^2	Thousand grain weight(g)	Number of grains cob^{-1}
T_1 -Control (RDF)	342.5	5	300.8	170
T_2 - Seed priming (SP)@ 1% $ZnSO_4$ solution	384.1	7	309.6	217
T_3 - Foliar application(FA) of 1% $ZnSO_4$ at 40 and 60 DAS	363.8	6	304.1	195
T_4 - Soil application(SA) of $ZnSO_4$ @ 25 kg ha^{-1}	446.4	8	323.5	289
T_5 - Soil application(SA) of $ZnSO_4$ @ 37.5 kg ha^{-1}	425.8	8	318.5	266
T_6 - SP @ 1% $ZnSO_4$ with FA of 1% $ZnSO_4$ at 40 and 60 DAS	404.9	7	314.2	242
T_7 – SP @ 1% $ZnSO_4$ with SA of @ 25 kg ha^{-1}	529.7	9	346.9	596
T_8- SP @ 1% $ZnSO_4$ with SA of @ 37.5 kg ha^{-1}	508.4	9	340.3	573
T_9- SP @ 1% $ZnSO_4$ with FA of 1% $ZnSO_4$ at 40 and 60 DAS Plus SA of $ZnSO_4$ @ 25kg ha^{-1}	487.8	8	337.0	549
T_{10}- Seed priming @ 1% $ZnSO_4$ with FA of 1% $ZnSO_4$ at 40 and 60 DAS Plus SA of $ZnSO_4$ @ 37.5kg ha^{-1}	466.9	8	332.1	314
SEd	9.3	0.18	7.42	9.80
CD(P= 0.05)	19.2	0.38	15.6	20.6

Table-2 Effect of seed priming with zinc sulphate on grain and biological yield (kg ha^{-1})

Treatment details	Grain yield(kg ha^{-1})	Biological yield(kg ha^{-1})
T_1 -Control (RDF)	2803	8100
T_2 - Seed priming (SP)@ 1% $ZnSO_4$ solution	3062	9193
T_3 - Foliar application(FA) of 1% $ZnSO_4$ at 40 and 60 DAS	3300	8687
T_4 - Soil application(SA) of $ZnSO_4$ @ 25 kg ha^{-1}	3994	10689
T_5 - Soil application(SA) of $ZnSO_4$ @ 37.5 kg ha^{-1}	3763	10194
T_6 - SP @ 1% $ZnSO_4$ with FA of 1% $ZnSO_4$ at 40 and 60 DAS	3535	9695
T_7 – SP @ 1% $ZnSO_4$ with SA of @ 25 kg ha^{-1}	4898	12627
T_8- SP @ 1% $ZnSO_4$ with SA of @ 37.5 kg ha^{-1}	4654	12172
T_9- SP @ 1% $ZnSO_4$ with FA of 1% $ZnSO_4$ at 40 and 60 DAS Plus SA of $ZnSO_4$ @ 25kg ha^{-1}	4438	11658
T_{10}- Seed priming @ 1% $ZnSO_4$ with FA of 1% $ZnSO_4$ at 40 and 60 DAS Plus SA of $ZnSO_4$ @ 37.5kg ha^{-1}	4224	11159
SEd	100	220
CD(P= 0.05)	210	462

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RESEARCH ARTICLE

A Review on the Volatile Compounds of *Capsicum Sp.* and Their Biological Activities

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ABSTRACT

Capsicum is a genus of flowering plants consisting of more than 30 species. It is one of the most commonly found items on the human diet and is consumed and cultivated all around the world. The fruit of most species of *Capsicum* contains capsaicin which is a lipophilic chemical. The amount of capsaicin also varies in different parts of the fruit and even on the climatic conditions and season during which it is being grown. 'Naga chilli' or 'BhootJolokia' (*Capsicum chinense* Jacq.) is a variety of chilli indigenous to the northeast region of India and has been recognized as one of the hottest chillies in the world. Apart from capsaicinoids other volatile oils such as Oleoresin are also reported in *Capsicum sp*. which contribute towards the aromatic part of the chilli. There are numerous volatile compounds reported from *Capsicum* which are involved in different biological activities. An insight into the biological activities of different Capsicum sp. is being discussed.

Keywords: Essential oils, Capsaicin, Oleoresin, Volatile compounds, GC-MS, Biological activity

INTRODUCTION

Essential oils are volatile secondary metabolites formed by aromatic plants and can generally be recognized by their characteristic odor [6]. They are natural aromatic oily liquids with complex compositions that are extracted from different parts of plants such as leaves, peels, barks, flowers, buds, seeds etc. Essential oils have been known to possess antioxidant and antimicrobial activities, and also can act as natural additives in foods and food products. They have great potential in the field of biomedicine as they effectively destroy several bacterial, fungal, and viral pathogens. Essential oils are composed basically of different types of aldehydes, phenolics, terpenes, and other antimicrobial compounds which is why essential oils are effective against a diverse range of pathogens. More than 3000 essential oils have been described in the existing literature, with approximately 300 in commercial use. The antimicrobial impacts of essential oils and their chemical components have been recognized by several researchers in the past. Furthermore, studies have shown the synergistic effect of any two or more ingredients of essential oils against various human pathogens [42]. Essential oils possess antifungal, antibacterial, and antiviral properties and have been screened on a global scale as potential sources of novel antimicrobial compounds and alternatives to treat infectious diseases. Therefore, essential oilscan serve as a powerful tool to reduce the bacterial resistance and greater attention has been paid to the screening of antimicrobial activity and its evaluation methods[12]. Medicinal plants, fruits and vegetables contain biologically active components with physiological and biochemical functions which play a key role in human. In recent years, antimicrobial properties of medicinal plants are being reported at a large scale. Dietary spices are important ingredients in Indian system of medicine. One of such dietary spice is the fruit of *Capsicum chinense*. It contains compounds known as capsaicinoids which causes the spicy flavour (pungency) of chilli pepper fruit [29]. The fruits are native to the North eastern part of India. It is known by various names such as "BhutJolokia" in Assam. BhutJolokia was earlier known as a hybrid variety of *Capsicum chinense*and *Capsicum frutescens*, but recently it has been reported as a distinct species, *Capsicum assamicum* [33]. According to the research done by Purkayastha et. al., *Capsicum assamicum* is most similar to *Capsicum chinense*and *Capsicum frutescens* but can be distinguished both morphologically and anatomically. Considering the importance of capsaicin in health and disease, and its commercial implications in the pharmaceutical and food industry *Capsicum sp.* offers great potential for future exploitation due to its high capsaicinoid content.

Species of *Capsicum*

Capsicum is a genus of flowering plants which belong to the family Solanaceae. It consists of more than 30 species, five of which are domesticated: *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens*[14]. The *Capsicum sp.* has a wide range of varieties which differ in shape, size, colour, taste as well as the pungency. The fruit of most species of *Capsicum* contains capsaicin which is a lipophilic chemical. The presence of this chemical is responsible for the pungency or the spiciness which is a commonly found trait in the capsicum species. The amount of capsaicin also varies in different parts of the fruit. It is present comparatively in less quantity in the flesh than the placental tissue where the seeds are found. The compound Capsaicin was first extracted in impure form in 1816 by Christian Friedrich Bucholz. He called it "capsicin", after the genus *Capsicum* from which it was extracted. Then in the year 1876 John Clough Thresh isolated capsaicin in almost pure form and gave it the name "capsaicin". Karl Micko isolated capsaicin in its pure form in 1898. The chemical composition of capsaicin was first determined by E. K. Nelson in the year 1919, who also partially elucidated capsaicin's chemical structure. Capsaicin was first synthesized in 1930 by Ernst Spath and Stephen F. Darling. In 1961, similar substances were isolated from chili peppers by the Japanese chemists S. Kosuge and Y. Inagaki, who named them capsaicinoids. The general biosynthetic pathway of capsaicin and other capsaicinoids was elucidated in the 1960s by Bennett and Kirby, and Leete and Louden. Through radio labeling studies the precursors to capsaicin is identified as phenylalanine and valine. *Capsicum chinense* is a species which includes different varieties endemic to different parts of the world. Most of the varieties of *Capsicum chinense* are known to have unique flavour and pungency. 'Naga chilli' or 'Bhoot Jolokia' (*Capsicum chinense* Jacq.) is a chilli variety indigenous to the northeast region of India and has been recognized as one of the hottest chillies in the world. This type of chilli has a very specific aroma. Different types of capsaicinoids including Capsaicin are found to be present in different Capsicum species which imparts the hotness to the chilli. Capsaicinoid content is variable in

various species. Out of them, *Capsicum chinense* Jacq. was found to contain the highest capsaicinoid content [25]. Apart from capsaicinoids other volatile oils such as Oleoresin are also reported in *Capsicum chinense* Jacq. which contribute towards the aromatic part of the chilli [13]. Oleoresins are semi-solid extracts composed of resin and essential or fatty oil, obtained by evaporation of the solvents used for their production. Essential oils of *Capsicum annuam* L. and *Capsicum frutescens* L. were reported to be extracted using hydro- distillation [18], [34]. However, there is dearth of work reporting antimicrobial activity of the aromatic part of *Capsicum chinense* Jacq although Capsicum has many ethno pharmacological applications including antimicrobial activities against various pathogens.

METHODS FOR THE EXTRACTION OF VOLATILE COMPOUNDS

Essential oil extraction

Essential oils represent a small fraction of a plant's composition consisting of numerous constituents, especially hydrocarbons and oxygenated compounds. It is important that the trace components are maintained during extraction of the essential oil so that the natural proportion of the components are intact. There are several methods for the extraction of essential oil. Some extraction method are best suited to particular plant types and parts. Although it seems relatively simple to isolate such oils, the composition of oil may vary to a large extent depending on the extraction method used [3], [10].

Distillation

Steam distillation or hydro-distillation is the most popular method used to extract and isolate essential oils from plants. The plant materials are boiled in steam or water so that the essential oil can be released through the process of evaporation. Under the influence of hot water and steam, the essential oil escapes from the oil glands in the plant tissue. As the steam and essential oil vapors are being condensed, they are collected and separated in a vessel usually called the ''Florentine flask" [16]. The extraction can be carried out by the use of Clevenger apparatus.

Solvent extraction

Solvent extraction can be used in the case of plant materials that are delicate aromatics or largely resinous that cannot withstand high temperatures which are used during distillation. During this method the plant materials are placed in baths of food grade solvents like hexane, ethanol, alcohol etc. to isolate the essential oils. After the plant material is being treated with the solvent, 'concrete' which is a waxy aromatic compound is produced. The concrete is then mixed with alcohol which initiates the release of the oil particles. This method is generally used in the production of perfumes by the perfume industry or for aromatherapy purposes [41].

Supercritical CO_2 extraction

In supercritical fluid extraction of essential oils, the constituents of the oils are not damaged by heat since it is usually performed at low temperatures. This is the reason why this is a very suitable method for thermally sensitive compounds. The main dissimilarity between distillation and supercritical extraction is temperature, i.e. in supercritical extraction CO_2 is used as a solvent instead of heated steam or water. Since this method uses CO_2 as a solvent it is environment friendly which causes no harm to neither the human body nor the environment. The oils produced through this method of extraction are of higher quality. In the year 1997 a paper was published by Piggott et al., on West Australian sandalwood oil where it was found that the extraction done using supercritical fluid extraction showed the highest yield of extracts and the overall volatile components [32].

Maceration

In this method carrier oils are used as solvents in the extraction process. The macerated oils are also called as infused oils. The extraction of essential oil through maceration ensures that more of the plant's essence is retained. It is hence beneficial than distilled oils. The plant materials used are made free of any moisture content which may cause harm to the final product [35].

Enfleurage

Enfleurage is one of the oldest methods of extraction of essential oil which is not commonly used now since it is time consuming and not cost efficient and also requires a lot of labour. It is used mainly for the extraction of essential oil from flowers. In this method of extraction odourless fats, either of animals or vegetables are implemented. This process can be done using heated as well as cold fat [41].

Cold-press extraction

This method of essential oils extraction is also known as Scarification or Expression. It is only used in the production of citrus oils in particular. Cold-press extraction is a physical process in which the essential oil glands in the peel are broken or crushed to release the oil. A device is used where the fruit is mechanically crushed and the essential oil along with the pigments are collected in the collection area of the device [8].

Simultaneous steam distillation and solvent extraction of volatile compounds:

Simultaneous distillation extraction (SDE) combines steam distillation with solvent extraction [21]. It is one of the most popular extraction methods. This method is also known as Likens–Nickerson steam distillation. It was first reported by Likens and Nickerson in the year 1964 and described in 1966 [31]. SDE performed using the Likens-Nickerson apparatus is a technique which is convenient for the isolation of volatile compounds. The SDE method has been considered as one of the most cited methods until now. Many researchers have successfully applied the SDE method for the extraction and analysis of various volatile compounds [44].

ANALYSIS OF THE VOLATILE COMPOUNDS

The analysis of the volatile compounds are done most commonly by using Gas chromatography-mass spectrometry (GC-MS). It is one of the most widely used techniques for analysing volatile organic compounds (VOCs) from plants. In the year 1994, Luninget, al., worked on the analysis of volatile compounds of fresh bell pepper using a GC-MS which was equipped with a thermal desorption unit [22]. Murakami et, al., analysed the volatile compounds from *Capsicum chinense* in a study published in 2019. For the analysis of volatile compounds, GC-MS and GC-time-of-flight (GC-TOF MS) analysis were performed and then its characterization was done [27].In the year 2015, Bogusz Junior et, al., published a research paper on analysis of volatile compounds in *Capsicum sp*. by headspace solid-phase microextraction and GC × GC-TOFMS. In the study a suitable method based on headspace solid-phase microextraction (HS-SPME) along with a time-of-flight mass spectrometry detector (GC × GC-TOFMS) and a chemometric approach was used to identify the characteristic compounds of each variety and assess the volatile compounds that could differentiate the samples[7].

BIOLOGICAL ACTIVITIES OF THE VOLATILE COMPOUNDS OF Capsicum

Researches show that essential oils exert activity in several microorganisms, presenting high application potential. Some authors have shown that spices and their derivatives, such as extracts, essential oils and isolated chemical compounds, have satisfactory results in inhibiting opportunistic pathogenic microorganisms, primary pathogens, deteriorating organisms, and/or inhibiting the production of microbial toxins. Several peppers have shown commercial interest due to this possibility of extraction of essential oils that have substances with antibacterial action, antifungal activities and insecticide in their properties and assist in the conservation process [23]. Natural antimicrobials from different sources are used to preserve food from spoilage and pathogenic microorganisms. Plants are the main source of antimicrobials and contain many essential oils that have preservation effect against different microorganisms [5]. The essential oil extracted from different spices were recorded to be showing microbial resistance. Many bacteria of food origin such as *Salmonella typhimurium, Bacillus cereus, Listeria monocytogenes* show pathogenic activity. These foods borne pathogens are reported to be killed by essential oils of different plants. There are reports on the usage of different essential oil extracts to kill different food borne pathogens. *Capsicum chinense* too exhibit antimicrobial property but there is much less work done showing the use of the essential oil of *Capsicum chinense* against food borne pathogens as well as its antimicrobial activity in general. Nagoth et al. studied the antimicrobial activity of capsaicinoids extracted from *Capsicum chinense* (BhutJolokia). The acetonitrile extracts of

Capsicum chinense fruits showed wide antibacterial activities against the human pathogens. Isolation of compounds having antibacterial activities could reduce the concentration needed to inhibit the growth of the microorganisms. Based on the antibacterial action, it was concluded that capsaicin present in *Capsicum chinense* fruits is very effective in the prevention of a lot of disease [29]. Roy, in 2016 published a review paper on *Capsicum chinense*. There is mention of a considerable amount of health benefits of capsaicin and dihydrocapsaicin found in BhutJolokia. There are also studies that showed wide antibacterial activities of the acetonitrile extracts of *Capsicum chinense*fruits against the human pathogens[36].In the year 2018, Salehi et al., studied on the potential phytopharmacy and food applications of *Capsicum spp*. It mentions various phytochemicals of *Capsicum spp.*

present antimicrobial activity and have been described as protectors against gastric pathogens. Chili inhibited the growth of foodborne gastrointestinal pathogens such as *Salmonella typhimurium*, *Listeria monocytogenes*, *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *S. aureus*, *E. coli* and *Vibrio cholera.* Moreover, isolated compounds from chili have been found effective against few types of pathogenic yeasts and fungi [37]. Sosa-Moguel et al. worked on the biological activities of volatile extracts from two varieties of *Capsicum chinenseJacq*, Mayapan and Jaguar varieties namely. The results of the study showed effective antibacterial and antioxidant activity. However in comparison, the Jaguar variety showed higher antioxidant and antibacterial activity than the Mayapan variety[39]. Among the volatile compounds, the following are being identified in *Capsicum sp*., thiol methane, dimethyl sulfide, dimethyl amine, acetaldehyde, propanal, acetone, 2-nonanone, hexane, acetic acid, 1-pentanol, limonene, pentadecane, ethyl ester propanoic acid, ethanol, 2-methyl-1-tetradecene, hexyl n-valerate, b-cubene, b-caryophyllene, butyrate, hexanal, a-pinene, linalool, limonene, hexyl isobutanoate, a-terpineol, methyl salicylate, heptylisopentanoate, decanoic acid, b-cubebene, germacrene D, 6-methyl-4-heptenyl 2-methylpropanoate, pentyl 4-methyl-2-pentanoate, ethyl 3-methylpentanoate, 2-butyl acetate, acopaene, byperene, a-humulene, g-cadinene, and acalacorene[4].

CONCLUSION

Antimicrobial agents have always carried major clinical significance, especially in this age of growing antibiotic resistance amongst common disease causing pathogens. The chemical composition of *Capsicum* genus is highly complex and rich which is why it can be applied in various fields. Several *Capsicum sp.* have shown commercial interest due to the possibility of extraction of essential oils and other volatile compounds that have substances with antibacterial action, antifungal activities, antioxidant activities, anticancer properties and insecticide in their properties. But the research done on this area is very limited and more work needs to be done to harvest the importance of essential oils of *Capsicum sp.*

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Table No. 01: Various biological activities of different volatile compounds of Capsicum.

Sl. No.	Sample	Volatile compounds identified	Biological activities	References
1	*Capsicum chinenseJacq.*	Hexyl 3-methylbutanoate, 3,3-dimethylcyclohexanol, hexyl 3-methylbutanoate, (Z)-3-hexenyl 3-methylbutanoate and heptyl 3-methylbutanoate	Antioxidant and antibacterial activity	[39]
2	*Capsicum annuum L.*	Hexanal, heptanal, 1-octen-3-ol, benzaldehyde, 6-methyl-5-hepten-2-one, octanal, (Z)-b-ocimene, (E)-2-	Antioxidant activity	[38]

		octenal, methyl benzoate, 2-isobutyl-3-methoxypyr-azine, menthol, ethyl octanoate, myrtenal, pentadecanal, myristicin, carotol, benzophenone.		
3	*Capsicum annuum*	trans-p-feruloyl-β-d-glucopyranoside, trans-p-sinapoyl-β-d-glucopyranoside, trans-p-ferulyl-alcohol-4-O-[6-(2-methyl-3-hydroxy-propionyl] glucopyranoside, luteolin and quercetin glycosides	Antioxidant activity	[2]
4	*Capsicum annuum*(Pepper horn)	9,12-octadecadienoic, linalyl acetate, Z, Z-10,12-hexadecadien-1-ol acetate, and 2-methyl-1,5-hexadiene-3-ol.	Antimicrobial activity	[2]
5	*Capsicum annuum*	hymol, hexyl isovalerate, linalool, D-Limonene, B-myrcene, Octanal, Y-Terpinene, 1-Octanol, hexyl isobutyrate, 1-Nonanol, 4-Carvomenthenol, Decanal, Terpineol, β-Citronnellol, Geranial, 1-Decanol, Thymol, Perilla alcohol, Decanoic acid, Copaene, β-elemene, 2,5-Dimethoxy-α-cimene, α-ionone, Caryophyllene, β-Farnesene, α-Humulene, Myristicin, β-Selinene, δ-Cadinene, Elemol, Elemicin, Spathulenol, Oxyde de caryophyllene, δ-Cadinol, α-Cadinol, Asarone, Myristic acid, benzyl benzoate	Antimicrobial activity	[15]

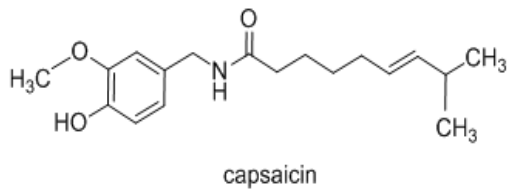

Figure No. 1: Structure of capsaicin

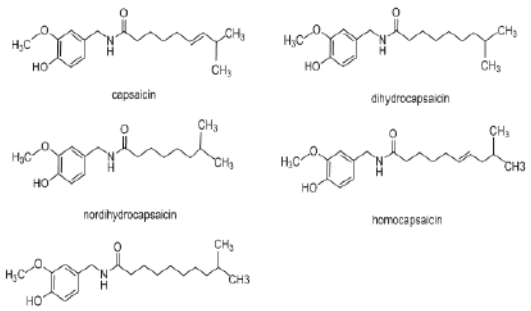

Figure No. 2: Compounds found in the oleoresin of Capsicum

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RESEARCH ARTICLE

Automatic Forest Fire Detection using Drones and Deep Learning Techniques

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ABSTRACT

Automatic fire detection is very important to detect and promptly extinguish fire in an earlier stage. There are ample studies investigating various fire detection techniques in existence. This paper implements ideas from various studies from the field of Image processing for fire detection, with a prototype drone as the final product for fire detection in various possible real-time scenarios. We use Image processing based forest fire detection, which includes converting the video feed to YCbCr color model, do background subtraction and draw contours on qualifying regions. The project is designed and executed in two phases, Hardware and Software. The hardware phase involves creation of a durable drone that can capture live video and relay it back to ground station. In the software phase, we start of by capturing live camera feed, applying Gaussian filter to the feed and convert the color space from BGR to HSV, applying background subtraction and contours to the fluctuating boundaries to detect the presence of fire and alert with an alarming system. We emulated the system camera feed on various stock videos. It was tested on videos covering fire confined to small areas, clearly distinguished from the background, fire not visible due to forest cover, only smoke is visible, fire spread to a very large area, with no forest cover remaining. The proposed system performs well in ideal scenarios.

Keywords: Drone, Forest fire detection, Image processing, Deep learning, CNN.

INTRODUCTION

Forest cover in India continues to ravage by the devastating fires in the forest. Forest fire is a natural calamity that leads to a huge loss of flora and fauna wealth of any country. This will also lead to the increase in the emission of carbon-di-oxide gas that makes our environment unhealthier. It has been reported a 125% increase in forest fire incidents between 2015 and 2017. The statistics report says that there are 345,989 forest fires from November 2020 to June 2021, according to the State of Forests Report, 2021 (SoFR, 2021), released in January 13, 2022. During November 2018 to June 2019, there were 258,480 forest fire incidents which shows the increase in the number [9]. Figure 1 shows the places of the forest fire alerts from April 1-14, 2021 in India as recorded by the Global Forest Watch, an open-source monitoring application. Hence there is a pressing need to identify and alert the government at an earlier stage of forest fire. Also, according to a recent estimate, India loses about Rs.550 crores every year because of the damage caused by forest fires [10]. Several decades of research have resulted in many advances in field of forest fire monitoring. This paper presents a reliable, simple solution that is less expensive for early stage fire detection. This paper covers a detailed literature survey on image processing used in fire detection, and also presents a detailed study on the fire detection algorithm using background subtraction and deep learning techniques. The system is also tested with various test inputs that covers a number of ideal scenarios. With the help of drones, we can easily monitor by capturing the images of the forest areas periodically and identify the places of fire and take quick actions so that the fire will not spread to a large extend.

LITERATURE REVIEW

A wide range of research is going on in the field of forest fire detection and mitigation. Some notable research papers that we came across while working on our prototype as inspiration and for knowledge are described below

Infrared image processing and its application to forest fire surveillance [1]

The work done by Bosch et. al focused on developing early fire detection using infrared imaging. They propose that the image processing be done off with the capture devices, so that computational power should not be a constraint. Novelty of their approach is incorporation of Optimal Statistical Signal Processing: i.e. provided a selected probability of false alarm, they are trying to maximize the probability of detection. They have two characteristics in their processing pipeline: persistence and increase(related to fire), thus, using statistical techniques and the mentioned two characteristics, they are able to keep the probability of false alarm under set threshold. Testing and simulation done by them has shown significant results, though they do discover that a number of false alarms emerge in cold and night conditions.

Identification of Forest Fire Based on Digital Image Processing [2]

Mahmoud *et al*. focused on the color characteristics of a fire image and validated that images captured from color camera sensors, instead of IR sensors, as being done by other studies, is also a feasible and cost effective approach. Their work revolves around YCbCr color space and the takeaway is their work on deciding the rules and thresholds for various color spaces. They use standard techniques like background subtraction and color space mapping from RGB to YCbCr, creating a simple yet effective processing pipeline. Their results of True Positive Rate of 91% and False Negative Rate of 12% establishes using RGB cameras instead of IR cameras as a viable solution for our problem, which has a major goal of being cost effective.

UAV-based Forest Fire Detection and Tracking Using Image Processing Techniques [3]

This paper, proposed by Chi Yuan et. al, elaborates the feasibility of unmanned aerial vehicles, for tasks like fire detection. They used the drones as a relay device, and did image processing offsite too, as is being done in other papers mentioned previously. A major portion of their work is around coordinating drones too, where they described how an alarm should be verified by peer drones from different angles. They have devised thresholds, mainly using experimentation, for the following criteria of an image snap of a moving video: luminance, chrominance of blacks, and chrominance of whites. They also introduce some morphological operations to remove

irrelevant objects from image stream, that we find useful for our task. Although we were not able to find tabulated statistics of their results, their results look promising and cost effective.

Image Processing Based Forest Fire Detection [4]

In the paper by Prema *et. al,* they have focused on experimenting and formalizing a processing pipeline, that is simple and effective, using if else cases of various thresholds in YCbCr color space. They do the image analysis in two steps: Identifying the fire vicinity, and then using another segmentation algorithm to find the source of fire in the initial segmentation. Their algorithm works on a static image, rather than a video, and uses thresholds of luminance that are defined by the them. The authors have put forward an accuracy of 99%, and a False positive rate of 12%. We find their approach to beless energy expensive and not easier to do on IoT devices.

Forest Monitoring and Wild land Early Fire Detection by a Hierarchical Wireless Sensor Network [5]

In this paper by Antonio et. al, the authors have experimented with mesh of sensors, that stay fixed at a place and send the data to the base station. They build upon the knowledge of estimates of probability of fire, which is calculated based on the moisture and other characteristic contents of the weather. Using this data, they can predict the ignition probability in the vicinity and stage an alarm. They are not using real time visual data as being used by other mainstream papers. The authors have tested their implementation with the local firefighting command centers during two fire simulations and it is a promising, novel and effective way to keep fires from spreading in larger areas before getting noticed.

Automatic Fire Detection: A Survey from Wireless Sensor Network Perspective [6]

This survey paper presents different ways and approaches to forest fire detection. Going through the automated ways of detecting fire, there was a pattern in all the approaches: almost all successful techniques are based on temporal contrast differences with relation to the natural background and/or the spatial characteristics due to the smoke cloud. The images are obtained either from a moving device or a static position, at a decent elevation to get maximum coverage. The detection algorithms usually send alarm messages with the co-ordinates of the location to the receiving station.

Automatic CO_2 Extinguisher Fire Fighting Drone [7]

Ethara *et. al* describe the software and hardware components required to develop a drone similar to our use case. They also give a quick overview of the forces that act on a quad copter frame, components, and telemetry. Their design is intended for usage in high-temperature environments, and everything is manually regulated at the ground station.

A Low-Cost Microwave Radiometer for the Detection of Fire in Forest Environments [8]

This paper describes how they have integrated different parts of a software to create a real-time fire surveillance and alarm system. Their software conforms to fire detection requirements they designed themselves using their literature survey. It does so by collecting telemetry data from IR cameras, evaluating the footage, completing the situation assessment, deciding the fire's geographic allocation, and finally generating a fire detection report for the control centre. The integration and networking, as well as how an ideal user interface for such software should look, are the primary takeaways for our problem statement.

EXISTING WORK

In the existing systems, mechanical devices or people are being used in traditional fire protection methods for monitoring the environment for forest fires. Techniques that use sensors employ particle sampling, temperature sampling, and airflow transparency tests for fire and smoke detection. In such a case, until the particles reach the sensors and activate them, an alarm is not triggered. The sensor's accuracy, reliability, and spatial distributions determine the system's performance. In the case of outdoor applications, vast numbers of sensors are required for high precision fire detection systems. Another popular research domain is automated fire-fighting. Prototypes in this

category are using water sprinklers, firefighting robots, and extinguishers, the current approaches aim to lessen the impact of fire. The automation is accomplished by employing thermo switches, and tubular type optical detectors. However, the extinguishers do not appear to reach the required potential, and is not feasible in large areas like forests.

MATERIALS AND METHODS

Hardware configuration

We have designed a basic drone with the specified hardware and software configurations as given in the subsections. The hardware architecture of various components and their connection is given in Figure 2. Table 1 gives the list of hardware components required.

Software configuration

• **Open CV:** Open CV (Open Source Computer Vision) is a library of programming functions that we are using for real-time computer vision. The library is cross-platform and free for use under the open-source BSD license.

• **Open Pilot:** Open Pilot is a GPLv3-licensed Open Source autopilot designed to fly multi-rotor aircraft including quad copters, helicopters and fixed-wing aircraft. We are using it to configure, control and monitor our drone.

Methodologies used

The fire detection module is built using two methods Image processing techniques (Method 1) and Deep learning model using CNN (Method 2).

Method 1 - Image Processing Methods

Method 1 is constructed in two phases.

• (M1-H) Hardware phase

• (M1-S) Software phase

(M1-H) The hardware phase

Involves creation of a durable drone that can capture live video and relay it back to ground station (laptop in our case). The basic drone prototype is given in Figure 3. We used off the shelf components and kept the complexity and number of components to the minimum to keep the drone light and reliable. The basic components used for the same are described in the Table 1. To keep the cost of the drone cheaper than the market alternatives and to increase the battery run time of the drone, we are doing the processing on the ground station rather than the drone itself. Also, we want to use minimum number of systems for as many drones as possible, so we need our implementation to be light weight and capable of real time detection.

(M1-S) In the software phase

We used Open CV to achieve the required objectives. The steps involved for fire detection are:

• (M1-S-1) Capture live camera feed

• (M1-S-2) Apply Gaussian filter to the feed

• (M1-S-3) Convert BGR to HSV color space

• (M1-S-4) Mask the frames to the neighborhood of HSV range of fire

• (M1-S-5) Background subtraction

• (M1-S-6) Apply contours to the fluctuating boundaries

The six steps are explained in detail in the following subsections.

M1-S-1-Capturing live feed from drone camera

The following steps are used for configuring Open-Pilot to receive the video feed captured by the FPV linked to the drone via an Analog receiver.

1. Connect CC3D to computer with a USB cable, open Open Pilot GCS, then click vehicle setup

wizard.
2. Upgrade the firmware if needed
3. Set default option (PWM) for input signal
4. Choose vehicle type, as Quad copter
5. Click Next to continue, and select the flight mode, here, choose X-model
6. Choose ECS mode, in this case we select rapid esc
7. Calibration - calculate and set board level
8. Calibrate the ESC signal
9. Connect the battery and the ESC
10. Follow on-screen instructions to do fine tuning and radio calibration.

M1-S-2-Applying Gaussian filter to the video

A Gaussian filter is a low-pass filter that removes high frequency components from an image using Gaussian blur applied to individual video frames. Gaussian blur is a type of image blur filter that uses a Gaussian function (statistical normal distribution)to calculate the transformation applied to each pixel of an image. Gaussian function in one dimension is represented in Equation 1.

$$G(x; y) = 1/2\pi\sigma^2 e^{-(x^2)/2(\sigma^2)} \quad (1)$$

In two dimensions, it is the product of two such Gaussian functions, meanwhile, in one in each dimension as shown in Equation 2.

$$G(x; y) = 1/2\pi\sigma^2 e^{-(x^2+y^2)/2(\sigma^2)} \quad (2)$$

where x is the distance from the origin on the horizontal axis, y is the distance from the origin on the vertical axis, and σ is the standard deviation of the Gaussian distribution. Applying this equation in two dimensions produces a surface whose contour is concentric with a Gaussian distribution from the center. The values from this distribution are used to create the convolution matrix that applies to the original image. The new value for each pixel is set to the weighted average near that pixel.

M1-S-3-Convert BGR to HSV color space

Hue, saturation, and value components are used to relate how people perceive color. The incoming feed is an RGB (red, green, blue) color model, which is suitable for applying masks to specific areas, so it is converted to an HSV (hue, saturation, value) color model.

The HSV colour space is represented as follows

$$V = \{x \mid x(H) \in [0, 359], x(S) \in [0, 255], x(V) \in [0, 255]\} \quad (3)$$

where x is a pixel in the HSV color space and x(H), x(S), x(V) are the H, S, and V component values of x respectively.

M1-S-4-Mask the frames to the neighborhood of HSV range of fire

We create three 2D projection planes from the HSV modeled video frames, i.e. sample fire colors are projected onto HS plane, HV plane and SV plane. Each plane specifies a range of color distributions, and anything that is not within that range is discarded, thus the resulting relative 2D color distribution is defined as

$$V_m = \{x \mid x(H) \in [a, b], x(S) \in [c, d], x(V) \in [e, f]\} \quad (4)$$

We obtain the segmented candidates for regions that contain fire, based on the color ranges of the three components obtained above.

M1-S-5-Background Subtraction

The segmentation method identifies images with wildfires, but may also get some false positives in the same color range, such as flowers, roofs, flags, etc. Therefore, in addition to color-based segmentation, we need to check the boundaries of such candidates. In the case of a fire, the boundaries will fluctuate, but in the case of false candidates, the boundaries will remain static. A library that uses a Gaussian mixed-based background / foreground segmentation algorithm was used to identify the foreground and remove the background. An important function of this algorithm is to choose the right number of Gaussian distributions for each pixel, because doing so improves adaptability to changes in the scene due to changes in lighting.

M1-S-6-Apply contours to the fluctuating boundaries

Contours are boundaries of shapes of the same strength and store the (x, y) coordinates of the boundary line of the shape of an object. After obtaining the fluctuating boundaries from background subtraction, we draw contours around the boundaries. If the area of the contour is larger than a threshold, the system flags it as fire and raises an alarm.

Method 2: Deep Learning model using CNN

We have used Convolutional Neural Network(CNN) to detect the fire in the video capture. The Algorithm1 is used for fire detection using a CNN framework:

Algorithm 1: Fire Detection Net

Input: Image

Output: Fire / Non-Fire

1. Collect the dataset for classifying fire and non-fire
2. The dataset is pruned for removing irrelevant extraneous images
3. Set training parameters, define - initial learning rate, batch size, no of training epochs
4. Develop a deep learning model Convolutional Neural Network used to detect smoke and fire in images - Fire Detection Net, use depth wise separable convolution
5. Build fire detection CNN model using Keras' Sequential API
6. Define CONV => RELU => POOL layers
7. Load the combined Fire and Non-fire dataset to instantiate the Fire Detection Net architecture.
8. Find optimal learning rate by using Learning Rate Finder class
9. Load the trained model, and test the model with some sample random images from dataset.
10. Classify and predict the label for each input image using the built model
11. Load and pre-process the image & Make prediction
12. Grab the highest probability label
13. Annotate the output class label in the top corner of the image
14. Save the output image to disk

Frame Capturing from input video feed

We capture frames continuously from the input video feed. Frame captured is tested for fire, using predict module, and the image is labelled as fire or non-fire. If the image is labelled fire for 10 continuous frames, beep sound with message 'Fire detected' displayed from the system. Else, if no fire is detected for 100 continuously captured frames, 'nothing detected' is displayed in the message box.

Implementation of Fire Detection Net using Convolutional Neural Network

The network we utilized uses depth-separable convolutions instead of standard convolutions as a depth-separable convolution. This way, the network requires less RAM and CPU to function, and is more efficient, as Edge/IoT devices will have limited resources and power draw. In some cases, it outperforms standard convolutions based approach and can improve the performance of fire / smoke alarms too. We used Keras' Sequential API to build our fire detection CNN.

Dataset Description

We have generated dataset with fire, smoke and non-fire images for constructing our fire detection model. The details are described as follows

Fire and smoke dataset

The dataset used to detect fire and smoke consists of a total of 1,315images searched by Google Image Search for searches related to terms such as "fire" and "smoke." The original dataset has irrelevant images that were not related to the fire or smoke (for example, a famous building before the fire). Those images have been removed in pre-processing.

Non-fire dataset

The dataset we used for Non-hearth place examples is referred to as eight-scenes and it consists of 2,688 photograph examples belonging to 8 different categories of herbal scene (all without fires). The dataset in the beginning was curated by Oliva and Torralba in their 2001 paper, "Modeling the form of the scene: a holistic illustration of the spatial envelope". The eight-scenes dataset is a herbal supplement to our fire/smoke dataset because it depicts herbal scenes as they must appearance without fire or smoke present. While this dataset has eight particular classes, we will consider the dataset as one single Non-fire class when we combine it with Fire dataset.

RESULTS AND DISCUSSION

We use the trained model and performed predictions on the frames captured from the sample video used for testing. The convolutional neural network used to detect smoke and fire was trained using two datasets. Once our network was trained we evaluated it on our testing set. On a random sample of 100images roughly 6 images were labelled wrong. The model was trained with the above mentioned dataset for a limited number of 50 epoch cycles to avoid over-fitting. Training accuracy at the end of the 50epoch cycles was found to be 87.74%. The initial approach used for fire detection using background subtraction and temporal variation offers a lesser accuracy.

We emulated the system camera feed on various stock videos. It was tested on videos covering three main areas:

1. Fire confined to small areas, clearly distinguished from the background.
2. Fire not visible due to forest cover, only smoke is visible.
3. Fire spread to a very large area, with no forest cover remaining.

The samples are given in Figures 4 to 6

CONCLUSION

We have demonstrated a prototype drone, built under a budget of Rs 20,000/- capable of capturing live aerial footage of forests, relaying it to a ground station which processes it in real time and detects if a forest fire is present. The system focused on building a fire detection module both using image processing and deep learning techniques. The latter was found to be more reliable with lesser false positives. The system can be used for early detection of forest fires, that could help in early mitigation and saving the precious forest flora and fauna.

The system works as expected in most of the scenarios, with the exception being:

1. In night time, if the fire is not visible and smoke is not illuminated or not able to see the smoke and thus fails to detect the fire till it catches a large area.
2. If the fire is indistinguishable from background.

Apart from the above scenarios, the system works as intended and utilizes minimal resources for its operations. The current system can be improved by adding thermal imaging and advanced smoke detection capabilities.

As a future extension, drones can be equipped with gas detectors (CxHx and H2 sensors) - to enable smoke detection. It can also be equipped with Microwave radiometer for smoke detection that can penetrate through thick green cover – with a radiometer generates 2.24 GHz frequency. Future research in fire/smoke recognition should focus less on computational intelligence architectures/training methods and more on the dataset gathering and curation process, ensuring that the dataset better represents how fires start, continue to burn, and spread in natural environments.

ACKNOWLEDGEMENT

We would like to thank the management of Sri SivasubramaniyaNadar College of Engineering for funding this project to develop a real time product which will be helpful to the society.

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Table 1: Hardware components used

S. no	Hardware component	Specification
1.	Flight Controller	CC3D
2.	Transmitter	2.4 GHz frequency
3.	Receiver	6 channels, RC832H 5.8G High Sensitivity Receiver
4.	Motors	B2212 920KV CW/CCW Brushless Motor For DJI Phantom
5.	Rotors	DJI 1045
6.	Battery	3S 30C/60C Lithium polymer battery Pack
7.	ESCs	30A Simon K ESC30A Program ESCs

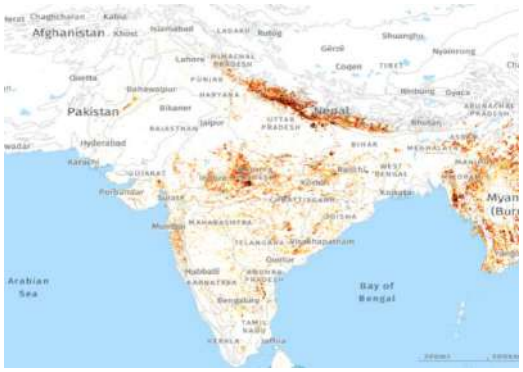

Fig.1:Forest fire alert places *source: downtoearth.org.in*

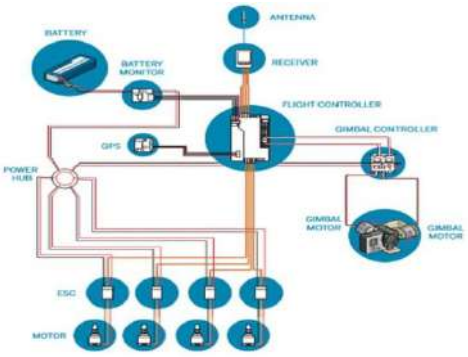

Fig. 2: System Architecture

Fig. 3: Basic built drone prototype

Fig. 4: Forest fire having fire behind forest covers

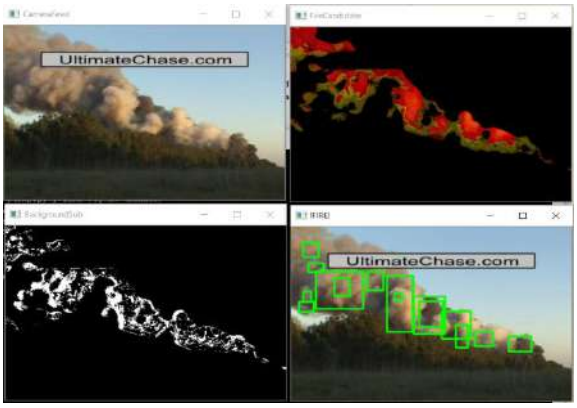

Fig. 5: Forest fire having visible smoke but no visible flames

Fig. 6: Forest fire having the fire spread to a very vast area with no forest cover

RESEARCH ARTICLE

Fourier Transform Infra Red (FT-IR) Spectral Studies of Polyherbal Ayurvedic Formulation of Anti-Psoriatic Drug Patoladi Kwatha Churna

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ABSTRACT

Pharmacological analytical investigations of medicinal plants continue to be a significant issue for analytical chemists. Because medicinal plants have a complex system of mixtures. The most popular methods of choice for the separation of active components and quality control of raw material and finished herbal products include gas chromatography (GC), high performance liquid chromatography (HPLC) and mass spectrometry (MS), which are the most popular methods of choice for the separation of active components and quality control of raw material and finished herbal products. Similarly, the application of FT-IR spectroscopy has also been used in herbal medication research but to a lesser extent. FT-IR spectroscopy was used to investigate the bioactive components present in patoladi kwatha churna, an anti-psoriatic Polyherbal Ayurvedic preparation. The constituents of six important crude pharmaceuticals are included in an ethanolic extract of Patoladi kwatha churna. Here's what they are: Trichosanthes dioica Roxb. leaves, *Picrorhiza kurroa royle ex Benth.*, rhizomes, *Santalum album Linn.,* heart wood, *Marsdenia tenacissima wight & Arn.,* root, *Tinospora cordifolia (willd) miers.,* stems, *Cissampelos pareira Linn.* roots.The results revealed that numerous biomolicules with a variety of pharmacological effects were deduced. This work looked at the prospect of using FT-IR spectroscopy to quickly identify a variety of functional groups responsible for therapeutic properties.

Keywords: FT-IR, Spectral Analysis, Polyherbal, Ayurvedic Formulation, Anti psoriatic drug, Patoladi Kwatha Churna.

INTRODUCTION [1,2,3]

Long before recorded history, Plants were employed for medical purposes. as early as 3,000 BC, ancient Chinese and Egyptian papyrus writings indicate medical benefits for plants. herbs were employed in healing rituals, by Indigenous civilizations (such as African and Native American), and herbal therapies were used in traditional medical systems (such as Siddha, Ayurveda, Unani and TCM). plant-based medications and other botanicals have become increasingly popular in the west in recent years. They have lasted the test of time because to their safety, efficacy, cultural acceptability, and lack of negative side effects. Memory loss, osteoporosis, osteoarthritis, diabetes, immunological and liver illnesses, and other age-related ailments for which no modern treatment or only palliative therapy is available are also mentioned in ancient literature. As early as 3,000 BC, Chinese and Egyptian papyrus literature reveal medical uses for plants. Herbs were employed in healing rituals by indigenous civilizations (such as African and Native American), and herbal therapies were used in traditional medical systems (such as Siddha, Ayurveda, Unani, and TCM). Plant-based medications and other botanicals have become increasingly popular in the West in recent years. They have lasted the test of time because to their safety, efficacy, cultural acceptability, and lack of negative side effects. Memory loss, osteoporosis, osteoarthritis, diabetes, immunological and liver illnesses, and other age-related ailments for which no modern treatment or only palliative therapy is available are also mentioned in ancient literature. Because the chemical elements in them are involved in the physiological process of living plants, they are thought to be more compatible with the human body. Over 1.5 million people practice traditional medicine which uses medicinal herbs for preventative, promotional and curative purposes. Due to the toxicity and side effects of allopathic medicines, the use of herbal drugs has resulted in a rapid expansion in the number of herbal drug makers.

Therapeutic uses of Patoladi Kvatha Churna In Ayurvedic Terms As Per AFI:[4,5,6]

Arocaka	-	Tastelessness
Chardi	-	Emesis
Jvara	-	Fever
Kamala	-	Jaundice
Kustha	-	Disease of the skin
Visa	-	Poison

A thorough literature survey on churnam used traditionally for skin disease activity was conducted in order to determine the churna that is traditionally used in the treatment of skin disease. Despite the fact that various churnas are used to treat skin diseases in Indian traditional medicine, the majority of them have not been clinically assessed. If a comprehensive and intensive ethnopharmacological investigation is conducted on one (or) more churnas employed in the traditional system, useful drugs for skin problems are certain to be produced.

Psoriasis [7,8,9]

Psoriasis is an autoimmune illness that causes patches of abnormal skin to appear on the skin. The skin areas are typically red, itchy, and scaly. Their intensity can range from minor and localized to a total body covered. The Koebner phenomenon is when an Injury to the skin causes psoriatic skin changes in that area.

Epidemiology [10,11]

Psoriasis is a skin condition that affects between 1% to 3% of the world's population. Men and women are equally affected.. Psoriasis has a bimodal distribution, with a peak between the ages of 15 and 20 and another peak between 55 and 60. Two forms of psoriasis have been postulated based on the bimodal distribution of age at onset and heriditary. Type I psoriasis (which accounts for around 65 percent of all cases) is linked to the beginning before the age of 40, a positive family history of psoriasis, a previous streptococcal sore throat, and guttae lesions. Type II psoriasis (which affects 35% of psoriasis patients) appears to be linked to a population beginning beyond the age of

40 and with no psoriasis in the family. There is no relationship between Type II and a previous viral trigger. Chronic plaques are the most common clinical picture, and a link with a nail and joint involvement has been reported.

Ayurvedic Drug Treated In Psoriasis [12]

Herbs can be used to treat psoriasis in a variety of ways, including dry extracts, tinctures, decoctions, topical creams, gels, and ointments, as well as oral formulations like tablets. These medicines offer significant anti-inflammatory properties similar to allopathic pharmaceuticals, with little or no negative effects even when used long-term.
The following are some of the herbs that have been used to cure psoriasis:

1. silybum marianum
2. rumex crispus
3. trifolium pretense
4. smilax sarsaparilla
5. coleus forskohli
6. stellaria medica
7. calendula officinalis
8. astragalus memranaceus
9. thespesia populnea
10. momordica charanta

TRADITIONAL SYSTEMIC TREATMENT

Ciclosporin [13]

Ciclosporin is an amino acid cyclic polypeptide of eleven amino acids. It inhibits lymphokine secretion (e.g., IL-2, IFN-, GM-CSF, IL-3, IL-4, TNF-, and IL-17) by suppressing the calcium-dependent phosphatase calcineurin, resulting in decreased T lymphocyte activation. Antigen-presenting cells are also inhibited by cyclosporin. Ciclosporin is a medication used to treat severe psoriasis. Since the emergence of biological medicines, their utilization has decreased in recent years. It does, however, have a role in situations where a quick effect is required. Ciclosporin is nephrotoxic, which means it can cause functional kidney impairment rapidly once treatment begins. Kidney function can be restored between treatment periods using intermittent therapies.Long-term treatment (more than two years) or ciclosporin doses of >5 mg/kg per day increase the risk of irreversible kidney impairment. Another side effect is hypertension, which can be treated by lowering the dose or starting an antihypertensive medication. Ciclosporin-treated patients who have previously received high doses of UV, particularly PUVA, are more likely to develop skin cancer, particularly SCC.

Acitretin [14]

Acitretin is an antiproliferative and immunomodulatory retinoid (synthetic vitamin A derivate). Acitretin inhibits the proliferation of epidermal keratinocytes and promotes their differentiation in the epidermis. Acitretin reduces the induction of Th17 cells while promoting T-regulatory cell development. Acitretin is used to treat plaque psoriasis, pustulous psoriasis, hyperkeratotic hand- and foot psoriasis, and erythrodermic (particularly when combined with UVB and PUVA). Hyperlipidemia and increased liver enzymes are the most common side effects.

Biologics [15]

Biologics are medications that are made from living organisms and affect the immune system. Psoriasis biologic treatments were first presented in Sweden in 2004. They are used to treat moderate to severe psoriasis when traditional systemic medications are contraindicated, can't be utilized because of adverse effects, or haven't produced adequate results. During therapy, there is a higher risk of serious infections, so tuberculosis and hepatitis screening is required before treatment begins. Although there is no strong evidence of a rise in the risk of cancer, the possibility of lymphoma or other cancers in the future cannot be ruled out.

Etanercept [16]

Etanercept is a soluble TNF receptor fusion protein that binds to circulating free TNF.

TNF-, which prevents TNF- from binding to TNF-receptors. It is subcutaneous injections are used for administration.

Adalimumab [17]
Adalimumab is an anti-TNF monoclonal antibody that is entirely human. Subcutaneous injections are used for administration.

Infliximab [18]
Infliximab is a human-mouse chimeric antibody that binds to both soluble TNF and TNF receptors.TNF acts on the cell wall and is given as an intravenous infusion.

Ustekinumab [19]
Ustekinumab is a human monoclonal antibody that binds to the p40 protein subunit of the interleukin (IL)-12 and IL-23 cytokines with high affinity and specificity. Subcutaneous injections are used to deliver it.

Preparation Of Extracts:
The whole plant was collected, dried in the shade, roughly powdered, and progressively extracted with ethanol using the soxhlet apparatus in a continuous percolation process. After the extraction process is completed, the product is concentrated using a rotating vacuum evaporator set to 60°C. The % yield was calculated after it was dried in a deep freezer at -40°C. The extract's appearance and consistency were also assessed.

MATERIALS AND METHODS [20,21,22]

The methods for identifying 'Herbal medications' are mostly aimed at obtaining a unique fingerprint of a certain plant that identifies the presence of a specific chemical ingredient. In contrast to FT-IR, various chromatographic techniques such as high-performance thin-layer chromatography (HPTLC), high-performance liquid chromatography (HPLC), gas chromatography,(GC), gas chromatography with mass spectrometry(GC-MS), and Liquid chromatography with high-resolution mass spectrometry,(LC-HRMS), have been widely used for this purpose. FT-IR has been widely used to discover phytoconstituents and elucidate structural elucidation in recent years. This approach is widely acknowledged in pharmacopeia since it receives fingerprint features and has broad applicability to samples. The goal of this work was to use FT-IR spectroscopy to identify the bioactive ingredients in the anti-psoriatic polyherbal formulation (PHF) patoladi kwatha churna (PKC). Phytochemical evaluation employs chemical assays to determine the nature of phytoconstituents present in the plant. The therapeutic activity of medicine is determined by the constituents present in the drug, hence phytochemical study is critical. It can be done using various chromatographic techniques such as TLC, HPTLC, GC-MS, LC-HRMS, HPLC, and so on. As a result, a thorough examination is necessary to characterize the phytoconstituents qualitatively and quantitatively.

Pkc Ingredients Collection and Authentification
Trichosanthesdioica Roxb leaves, Picrorhiza KurroaRoyle ex Benth rhizomes, *Santalum album* Linn heartwood, Marsdenia Tenacissima Wight and Arn roots, *Tinospora cordifolia* (willd) Miers stems, and *Cissampelos pareira* Linn roots were all gathered from different locations of India. Dr.P.Sathyarajeswaran, Assistant Director (Scientist -2), Central Siddha Research Institute, Govt. of India, Arumbakkam, Chennai, confirmed the authenticity of the three plants. Dr.R.Ilavarasan, Assistant Director (Scientist -3) Incharge, Regional Ayurveda Drug Development Institute, Ministry of Ayush, Govt. of India, Arumbakkam, Chennai, also confirmed the authenticity of the three plants.

Sample Preparation
For IR Spectroscopy, 1 mg of solid sample was combined with 3 mg of potassium bromide and dried in an oven at 120°C for overnight before being compacted into a thin clear pellet using a hydraulic press under 10 t/cm2 pressure and 20 mbar vacuum. Place the pellet immediately in the FT-IR spectrometer's standard sample mount. The spectrum was measured using a Perkin Elmer Spectrum one model FT-IR instrument with a wavelength range of

4000cm-1 to 400cm-1 and a resolution of 4cm-1 over 32 scans. The spectra were recorded at room temperature using KBr pellets as a standard after the dried material was milled with KBr and pressed to form pellets. Advanced Research in Indian System of Medicine (Carism), Sastra Deemed University, NH67, Thirumalaisamudram, Thanjavur, conducted the research.

RESULTS AND DISCUSSION

The numerous chemical constituents of Patoladi kwatha churna of anti-psoriatic medication have been discovered using FT-IR spectrum investigations. The FT-IR spectrum was recorded, as well as the spectral data indicated previously (Figure-1). The FT-IR analysis of each component's peak levels is mentioned in the report (Table-1).

1. In primary alcohol, the band at 4000 cm-1 is set aside for O-H stretch.
2. In strong broad alcohol intermolecular bonded, the band at 3400.88 cm-1 is allocated for the O-H stretch.
3. In alkanes, the band at 2925.31 cm-1 is reserved for the C-H stretch.
4. In carboxylic acid, the band at 1711.67 cm-1 is reserved for a significant C-O stretch.
5. In alkene, the band at 1635.22 cm-1 is designated for medium C=C stretch.

The band at 1515.21 cm-1 is reserved for the nitro compounds with a significant N-O stretch.

7. In alkane, the band at 1383.88 cm-1 is designated for medium C-H bending.
8. The band at 1281.39 cm-1 is reserved for aromatic amines with a significant C-N stretch.
9. The band at 1073.13 cm-1 is set aside for primary alcohol with a strong C-O stretch.
10. In aliphatic chloro compounds, the band at 768.67 cm-1 is reserved for significant C-Cl stretch.
11. Strong C-l stretch in aliphatic iodo compounds is assigned to the band at 592.59 cm-1

The presence of the above data indicates that various functional groups such as primary alcohol, alkenes, alkanes, aromatic amine, nitro compound, aliphatic chloro, and Iodo compounds have been reported for various medicinal properties such as antimicrobial, antitumor, immunomodulatory, hepatoprotective, blood purifier, skin diseases, and anti-inflammatory.

CONCLUSION

This work looked at the prospect of using FT-IR spectroscopy to quickly identify a variety of functional groups responsible for therapeutic properties. The inclusion of functional groups such as primary alcohol, alcohols, alkanes, carboxylic acid, alkenes, nitro compounds, alkanes, aromatic amines, primary alcohols, aliphatic chloro compounds, and aliphatic iodo compounds contribute to the medicinal characteristics of this herbal composition. Further research into the bioactive molecules found in this polyherbalayurvedic formulation could lead to the discovery of additional bioactive compounds.

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Table- 1 Composition of Patoladi Kwatha Churna (PKC)[4,5,6].

S.No	Tamil Name of the Plant	Biological Source	Quantity
1	Kambu pudalai	*Trichosanthes dioica* Roxb. Using parts - Leaves	5g
2	Kadugurohini	*Picrorhizakurroa royle ex* Benth Using parts - Rhizome	5g

3	Chandanam	*Santalum album* Linn Using parts – Heart Wood	5g
4	Perunkurinjan	*Marsdenia tenacissima* Wight and Arn Using parts - Roots	5g
5	Seenthilkodi	*Tinospora cordifolia* (Willd) Miers Using parts - Stem	5g
6	Vattatiruppi	*Cissampelos pareira* Linn Using parts - Roots	5g

Table-2:

Position-(Cm^{-1})	Intensity	Bond	Functional groups
4000	29.56	O-H stretch	Primary alcohol
3400.88	9.17	O-H stretch	Alcohols
2925.31	9.96	C-H stretch	Alkanes
1711.67	14.69	C-O stretches	Carboxylic acid
1635.22	16.52	C=C stretch alkene.	Alkenes
1515.21	19.27	N-O stretch	Nitro compounds
1383.88	16.36	C-H bending stretch	Alkanes
1281.39	15.83	C-N stretch	Aromatic amines
1073.13	14.00	C-O stretch	Primary alcohols
768.67	26.59	C-Cl stretch	Aliphatic chloro compounds
592.59	25.39	C-l stretch	Aliphatic iodo compounds

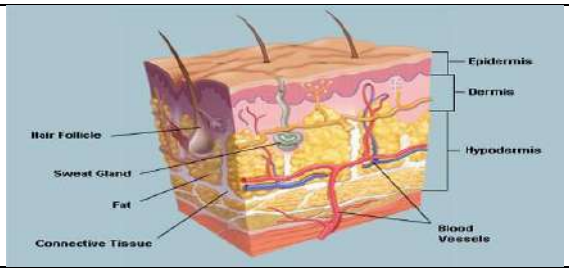

Fig. 1. Normal Skin

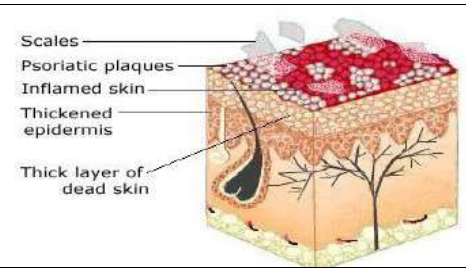

Fig. 2. Psoriasis Skin

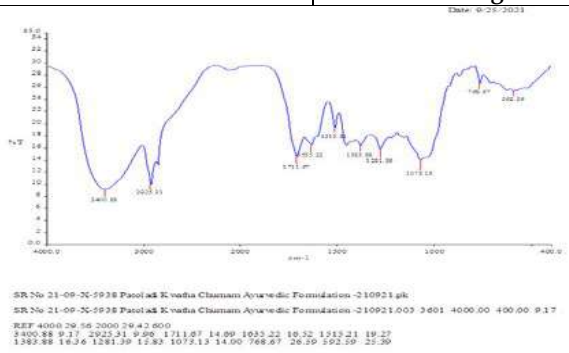

Fig. 3. The FR-IR Spectra Data of Patoladi Kwatha Churna of Antipsoriatic Drug

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REVIEW ARTICLE

Impact of Farm Yard Manure on Soil Quality: An Overview

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ABSTRACT

Soil health is the ability of the soil to provide an environment for optimum growth and development of plants, while also ensuring the health of animals and human beings. Livestock waste as farm yard manure has been used for centuries as a source of fertilizer for improving the soil fertility. Farm yard manure application affects water holding capacity, soil fertility and other biological properties to some extent. This was to compile the research findings on the effects of various livestock manure types on soil fertility, soil physical properties, soil biology and the yield of various cereal crops. Specifically, this paper summarizes results for poultry and cattle manure used in various cropping systems. Inspite of the fact that there are conflicting results in the literature with regards to the effect of farm yard manure on different soil properties, they offer persuading evidence of useful impacts of manure on soil and the crops. The degree to which manure affects soil depends on the physical and chemical properties of the manure itself and various management and environmental factors including rate and timing of application, soil type and climate. It indirectly encourages the farmers to include livestock components which enable them to passive doubling of income.

Keywords: farm yard manure, soil fertility, nutrients, soil quality

INTRODUCTION

Farm yard manure was applied to crops as a fertilizer since the early years of agricultural development in India. Earlier the government and NGO's created the awareness of the nutrient value of farm yard manure among farmers. They also sought to encourage the farmers to use farm yard manure rather than completely replacing it with commercial fertilizers. Economic and demographic developments after the World War II brought about an increase in agricultural production efficiency which resulted in the rise of large concentrations of livestock operations at the same time that commercial fertilizer production was also increasing to improve the productivity of the available land

to feed the growing population. As of now, soil health deterioration as a result of erosion, desertification, tillage and unsustainable agricultural practices have caused a significant decline in productivity on some land [1]. On the other hand, the exponential growth in world population has expanded food demand, which requires higher production. These developments require the usage of practices that improve or reestablish the quality of land. Manure has been known to have advantageous effects on soil fertility and numerous other soil properties, contributing to the by and large soil wellbeing. Soil quality may be defined as the capacity of the soil to be a vital living ecosystem continuously that supports plants, animals and humans [2]. One of the reasons that there has been an increasing trend in the use of farm yard manure is the fact that they are a source of carbon which plays a role in improving soil quality. Increased awareness on organic food and consumer's interest also contributed to the use of manure as fertilizer [3, 4].The manure types that are most used on a global scale was identified. These manure types included cattle, poultry and swine manure. Some of the soil quality indicators that are taken into account in this study were total nitrogen and nitrate, soil pH, water holding capacity, Soil Organic Matter and Carbon**,** soil temperature and soil microbial status.

SOIL NUTRIENT STATUS

Soil fertility is defined as the available nutrient status of the soil and its ability to provide nutrients inherently and from external sources [5]. Different studies have reported an increase in macro and micronutrients as a result of manure application [6-8], which in turn positively affects the growth and productivity of crops. The nutrient content of manure depends on several factors including animal type, feed intake and water consumption by the animals [4], manure storage and management.

TOTAL NITROGEN AND NITRATE

A study conducted by Hou *et al*. [10] showed that the application of poultry manure in combination with inorganic fertilizer significantly increased the N content in plant parts. Conversely, manure application has been associated with increased nitrate (NO_3) leaching from soils [11]. The dependence on environmental factors such as moisture and temperature and the potential losses make the availability of N from manure highly variable and unpredictable. As a result, producers often over apply manure to land which in turn becomes a potential problem to the environment. This paves the highest contribution of FYM in terms of Nitrogen content. Another study showed that increasing the poultry manure application rate from 5 to 10 Mg per ha did not cause a significant increase in total soil N [9]. These findings confirm the unpredictability of the release of nutrients from manure.

FYM AND ITS INFLUENCE ON SOIL WATER AND SOIL HYDRAULIC PROPERTIES

One of the challenges we face in agriculture is the scarcity of water [13]. According to Mekonnen and Hoekstra [14], an estimated 4 billion people live with severe water scarcity at least one month out of the year. With the growing world population and the increase in food demand, the pressure on this already scarce resource will only increase. One of the ways to mitigate this problem is to increase on-farm water retention which can be accomplished by applying organic soil amendments to land. Various properties of the organic matter itself, however, may contribute to the increase in soil water retention. Soil water holding capacity or available water capacity is the amount of water that a soil can hold for use by plants. Ahmed et al. [17] found that soil treated with poultry or farmyard manure retained more water than untreated soil. Similarly, Nyamangara et al. [16] found that the addition of cattle manure to soil increased water retention in comparison to the control treatment where no manure had been applied. In a study evaluating the effect of farmyard manure on soil physical and chemical parameters, SchjØnning et al. [12] found a higher water retention for farmyard manure compared to NPK fertilizer and unfertilized treatments at depths of 8-12 cm and 30-35 cm. In an early study by Bouyoucos [15] it was shown that adding 54 Mg of partially decomposed horse-cow manure to a sandy loam increased the percent by volume soil water content by 10.2 percentage points relative to the plots were no manure had been applied. These findings suggest that manure application affects soil water. The increase in soil water retention as a function of manure application is likely an effect of the organic matter on soil porosity. Thus it becomes evident that Cattle, Horse and Poultry manure has a positive influence on soil water holding and hydraulic properties.

SOIL TEMPERATURE

Soil temperature and temperature fluctuations affect various soil health indicators. In a study evaluating the effect of poultry manure on various soil properties, Agbede et al. [18] found that the addition of 7.5 Mg ha^{-1} manure consistently decreased the soil temperature by 2 to 2.3 degrees Celsius. The lower temperatures associated with the application of organic amendments is likely caused by improved water retention and protection of the soil against large temperature fluctuations [19]. Similarly, Unger and Stewart [20] showed that the addition of farmyard manure resulted in a reduction in evaporation, a direct effect of manure on soil physical properties. Evaporation has a cooling effect on the soil surface, thus a reduction in evaporation suppresses the cooling of the soil surface.

SOIL MICROBIAL STATUS

Improving soil health requires a holistic approach that does not only supply nutrients in adequate and balanced amounts but also enhances the soil biological system. In particular, soil microorganisms are a hallmark of soil as a living system that in some instances solely dictates the rate of reactions that takes place during nutrient cycling [21]. In studies examining the role of farm yard manure in soil fertility, Parham et al. [22] and Hamm et al. [23] demonstrated that manure application enhances the bacterial community in the soil, thus leading to an improvement in soil productivity. These studies found bacteria to thrive well and grow large in population size in the soil treated with livestock manure. Regardless of the dominant microorganisms, this review demonstrates that applying manure is invaluable for improving soil fertility by increasing the population of microorganisms that are useful for nutrient transformations in the soil. Thus it can be culminated that soil microbial count and fertility can be exclusively enhanced by livestock manure.

SUMMARY

Soil health is a broad term that speaks to the capacity of the soil to function as an ecosystem that supports the plant, animal, and human life. This review shows that manure contributes to creating this ecosystem in supplying nutrients and improving various soil properties. Improved soil fertility, water movement and retention, and soil temperature regulation do facilitate better growth and higher productivity of crops. The large amounts of manure that is applied on the land prevents the synthetic fertilizers and paves way for organic farming. To cut a long story short, this paper summarizes the importance of FYM on soil fertility and water retension capacity. It also enables farmers to follow organic farming thus providing a viable alternative for traditional farming techniques and prevents health hazards of food cycle. It also encourages farmers to raise livestock thereby helping to double their income by way of integrated farming system.

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RESEARCH ARTICLE

Construction and Standardization of Parental Involvement Scale

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ABSTRACT

The purpose of this paper was to construct and evaluate a Parental Involvement scale. For standardization of this scale, it was needed to assess the validity, reliability, and suitable standards for interpreting the results of the scale. There are three sub-scales (dimensions) with fifty-eight (58) items/statements that make up the final form of the scale. Experts in related domains helped to validate the scale's content. The construct validity for items was determined using factor analysis, and the discrimination validity for each dimension was determined using a t-test for two independent groups (one upper group with 27% and another lower group **with** 27%, respectively). Alpha coefficients were used to determine the overall scale's and each dimension's dependability. It may be determined from the reliability coefficients that indicated the scale was reliable because all three dimensions' alpha coefficients values were more than. 70. As a final point, the relevant parameters for interpreting stanine procedures scores were addressed. The stanine process is a systematic method for categorizing scores to understand Likert scale replies in a meaningful way. However, in order to interpret the scores using stanine procedures, the investigator gathered sample raw data and after collecting raw data from all of compute the z values of the raw scores.

Keywords: Parental Involvement, Tool Construction, Evaluation, Factor Analysis, Likert Scale, Reliability, Validity, Stanine Procedure

INTRODUCTION

Several tools had been created to assess parental involvement in both foreign and Indian contexts. Few foreign scales had been developed by A. S. Wilkins, Joan M. T. Walker, James R. Dallaire, Howard M. Sandler and Kathleen V. Hoover-Dempsey (November 2005), Heather Marie Scott (2011), Abbie Solish, Adrienne Perry, Rebecca Shine (November 2015) and some Indian scales such as Rita Chopra and Surabala Sahoo (2007), Naved Iqbal (2014), etc. Although we all know, foreign scales were not culturally appropriate, making them inappropriate for usage in the Indian context. Another problem with using foreign scales was unreliable when used with adolescents. Another difficulty was their use of the language of the things for which they were originally designed, as well as their failure to measure all of the key dimensions globally acceptable in which Parental Involvement as a predictor for self-regulated learning and academic achievement. Likewise, Indian tools evaluating parental involvement are not without flaws, such as the evaluation of self-regulated learning and academic achievement for the middle age group, Hoover-Dempsey (2005). Parents' educational involvement has been described in a variety of manners, but it has constantly been demonstrated to have a favorable effect on students' accomplishments, regardless of its form.

For instance, parental expectations and whether parents regard performance have a favorable relationship with children's academic achievement (Seginer, 1983, 1986). (Paulson, 1994; Steinberg, Lamborn, Dornbusch, & Darling, 1992). Furthermore, a study had shown that parental participation in their young one's schoolwork or homework, as well as their involvement in school events and co-curricular activities, has a good association with academic achievement (Stevenson & Baker, 1987; Steinberg et al., 1992; Paulson, 1994). Most of the studies' focal point was on parental involvement in Primary school students. The presumption was that parental involvement reduced when children move to secondary school and senior secondary school because they believed that they couldn't support their adolescents with more challenging high school subjects (Hill & Chao, 2009). The secondary school system is another roadblock, as it discourages parental involvement. Due to the obvious size and complexity of middle schools, it's difficult for parents to know who to contact for information about their children's progress (Sanders & Epstein, 2000). Parents communicate with teachers who teach a big number of students and consequently communicate with students learning less frequently in the secondary school system (Eccles & Harold, 1996). On the other hand, educational and developmental studies, emphasize the significance of parental involvement in their children's education. Particularly, parents remain a significant pillar of help for adolescent children (Laursen and Collins 2004), and their educational involvement has an impact on their children's academic motivation and achievement (Seginer, 2006). The purpose of this study was to investigate the direct and indirect effects of parents' educational participation on adolescents' academic attainment. This research examines a three-dimension framework in which adolescents' perceptions of parental participation are linked to self-evaluation, which is related to school-reported academic accomplishment. To find out this framework the researcher adopted the two dimensions' approach with the help of previous standardized tools and explored one more dimension of responsibilities for learning outcomes which was related to parental encouragement, adolescence self-worth, and self-evaluation.

Objectives

- To construct and evaluate the Parental involvement scale for senior secondary school students with special reference to academic achievement.
- To evaluate the validity of the Parental Involvement scale for senior secondary school students.
- To assess the reliability of the Parental involvement scale for senior secondary school students.
- To identify suitable guidelines for interpreting the findings of the Parental Involvement Scale.

DESIGN OF THE STUDY
METHODOLOGY

In this present study, the descriptive statistical method was applied. The descriptive survey method was used for collecting, tabulating, and interpreting the meaningful data.

Sampling technique

The study's samples include 100 school students presently registered in class 9th of several (Government/Private) schools in West UP of G.B. Nagar in October 2021. This research was limited to students in 9th grade. Second, the population's candidates range in age from 14 to 15 years.

Stages of tool construction

There were no universal laws among experts on the particular techniques for scale construction. However, to assure the quality of a tool, it must go through some stages throughout its construction (Alderson, 1995). Although adequate tool construction procedures are required.

Preparation of the preliminary draft

Following several literature reviews and prior scales, as described in the introduction, three dimensions were chosen based on the three mentioned parameters that are connected to total parental educational involvement. This parameter includes dimension as parental home-based involvements and parental school-based involvements. Home-based parental involvement techniques refer to parental educational practices with their children at home, while school-based parental involvement tactics include parental participation in volunteer activities at school, communication between parents and teachers regarding their children's performance, and participation in school governance (Epstein, 1987; Conners & Epstein, 1995; Epstein & Sanders, 2002). Home-based parental involvement is defined by Scott-Jones (1995) and Seginer (2006) as education-related activities that take place at home and apply to three aspects: first, motivational (such as providing support and setting standards of achievement), second, cognitive (such as teaching the child to read and solve logical or mathematical problems), and third, behavioral (such as teaching school-related routines, especially those related to school conduct and schoolwork). School-based parental involvement entails extracurricular activities such as assisting the school in a variety of areas, ranging from academics to extracurricular. Thus the items correlated with the three dimensions which were taken, one by one, according to the nature of each dimension. Previous tools and researches on parental involvement, as well as the relevant literature, were used to choose the items. When considering items, the nature of the item and the intended dimensions of parental involvement were properly considered. In this manner, the initial draft was prepared and 58 items (25 items on home-based parental involvement, 22 items on school-based parental involvement, and 11 items on responsibilities of parents towards learning outcomes) were comprised in this scale. The drafted items were then delivered to specialists from several universities with extensive knowledge in the related area of scale construction, with the appeal that they studied the drafted items and evaluated their content correctness coverage, editorial quality, and ideas for item additions, deletions, and modifications. Consequently, 60% universal consent, 23 items were eliminated and 35 items were kept, as follows given below in table 1:

Try-out of the tool

In this draft, 35 items were administered to 100 samples of school students at the senior secondary level from G.B. Nagar (UP). This is a Parental Involvement scale measuring the adolescents' academic achievement towards their Parental Involvement. This scale required those adolescents who tell the Parental Involvement as a predictor of self-regulated learning and academic achievement. For each statement of the scale, there are five options: "Strongly Agree," "Agree," "Undecided," "Disagree," and "Strongly Disagree." 'Strongly Agree' received 5 points, 'Agree' received 4 points, 'Undecided' received 3 points, "Disagree" received 2 points, and "Strongly Disagree" received 1 point. The marks that were awarded to the A, B and C dimension statements were totaled and summed to produce the overall composite score on that dimension.

Item Analysis

To refine the scale, "Cronbach alpha" was utilized to examine the item analysis. The data was gathered and analyzed. The overall score of the tool was correlated with the individual item scores. The Item Vs Whole correlation method was used to do item analysis on the 100 response sheets. Total scores of each dimension were computed. After that t-tests were calculated by the individual item of 27% upper case group and 27% lower case group the corresponding item score. Items were selected at the 52 Degree of freedom. When the degree of freedom is 100, the correlation

coefficient is 1.96 at the 0.05 level of significance and 2.58 at the 0.01 level of significance (Best, J. W. 2006). As a consequence, items with "t-test" values of 1.96 or above were selected. It was observed that 35 out of the 58 items showed significant positive correlations with the overall score of the scale, excluding 23 items that have no substantial correlation with the total score of the scale. The computed t-test table was shown below: Table 2 shows that 23 items (bold and italic) didn't have a significant correlation with the scale's overall scores so these items were removed from the scale. After removing these 23 items, the rest 35 items were chosen, which were related to three dimensions of the parental involvement scale.

Tool validity evaluation

Validity refers to a test's ability to measure what it was designed to measure. To see the test validity, the researcher inspected face validity, construct validity, and discrimination validity.

Face validity

Experts evaluated the face validity of the "Parental Involvement Scale." Experts' assessments indicated that these items were directly connected to three dimensions of the parental involvement scale.

Construct validity

To evaluate construct validity, the investigator calculated the correlation between each subscale score and the scale's overall score. Based on the information in table 4, it was possible to interpret that the correlation coefficients for all dimensions (0.320, 300, and 0.554, respectively) were significant at 0.01level. It was suggested that each dimension of Parental Involvement was correlated and the tool has good construct validity.

Factor Analysis

Although, factor analysis requires a minimum of 100 samples. The parental involvement scale has been submitted to exploratory factor analysis (Kline, 1986). In this scale, all three dimensions were kept. According to the exploratory factor analysis, all of the items are measuring the same construct.

Discrimination validity

The researcher applied item analysis to determine the discrimination validity of the items to find the level of difficulty value and level of discrimination value. The discrimination value of each dimension of parental involvement was determined using the 't' test for two independent samples (upper group 27% and lower group 27%). Ultimately, the 't' test was used to assess the discrimination validity of the entire test. The below table shows the discrimination validity of each item of the entire test. It shows that 't' values of the scale were significant at level 0.01, and the upper group's mean score was also higher than the lower group of the mean score. It indicated that the Parental Involvement scale has good validity.

Reliability of the Parental Involvement Scale

Reliability refers to the degree of consistency among findings. For each dimension of the full scale, the reliability coefficients (Cronbach alpha) are listed below:

Finalized draft

The finalized draft of the scale, including the serial number of items, is shown below:

RESULTS AND DISCUSSIONS

The following findings were reached after properly applied statistical techniques to construct the scale. The current study developed a scale that measures senior secondary school student's academic success in relation to their parental involvement. The scale consists of 35 items that were used to measure three dimensions of Parental

Involvement viz, Home-based parental involvement, School-based parental involvement, and Responsibilities for learning outcomes.

- The scale's content, construct, and discrimination validity have been validated. The discrimination validity was assessed using the 't' test, with the upper group scoring 27% and the lower group scoring 27%. Overall 't' values were significant at 0.01 level and the means of the upper group were significantly greater than the lower group, indicating that the Parental Involvement has high validity.
- The Cronbach Alpha Coefficient was used to measure the reliability of the scale. All the values of reliability coefficients were more than 0.70. The reliability of the Parental Involvement scale was 0.900, and the reliability of each dimension of Parental Involvement was 0.917, 0.900, and 0.923 respectively.
- The researchers used the stanine procedures with respect to their academic achievement towards Parental Involvement.

CONCLUSION

Parental involvement, that is "parents' interactions with schools and with their children to promote academic success" (Hill and Taylor 2004, p. 1491), is an umbrella term that includes a variety of behaviors and activities of parents directly or indirectly related to the education of their children. Several researches have been conducted over the years that support the fact that there is connection between parental involvement and student academic success, learning outcomes, and motivation.

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Table 1: Three dimensions of parental involvement with the selected number items

S. No.	Dimensions	No. of Items
A	Home-based parental involvement	13
B	School-based parental involvement	14
C	Responsbilities for learning outcomes	8

Table 2: Computed 't'-value table

Item	Mean	Std. Deviation	N
H1	***4.46***	***0.874***	***52***
H2	***3.83***	***1.53***	***52***
H3	4.29	0.871	52
H4	4.46	0.959	52
H5	***3.08***	***1.412***	***52***
H6	3.35	1.545	52
H7	***3.65***	***1.297***	***52***
H8	3.31	1.541	52
H9	4	1.252	52
H10	***3.08***	***1.713***	***52***
H11	3.98	1.336	52
H12	***3.79***	***1.486***	***52***
H13	***4.21***	***1.073***	***52***
H14	***4.42***	***1.054***	***52***
H15	***4.25***	***1.118***	***52***
H16	4.02	1.407	52
H17	4.37	1.01	52
H18	4.19	0.971	52
H19	***3.67***	***1.396***	***52***
H20	4.13	1.221	52
H21	4.13	1.03	52
H22	***3.79***	***1.513***	***52***
H23	***3.92***	***1.266***	***52***
H24	3.88	1.215	52
H25	3.62	1.561	52
S1	***3.57***	***1.5***	***52***
S2	3.66	1.544	52
S3	4	1.127	52
S4	3.75	1.453	52
S5	4.17	0.955	52
S6	***3.72***	***1.321***	***52***
S7	***4.25***	***1.175***	***52***
S8	***3.87***	***1.532***	***52***
S9	4	1.225	52
S10	***3.72***	***1.561***	***52***
S11	3.15	1.499	52
S12	***3.13***	***1.582***	***52***
S13	3.77	1.54	52
S14	3.89	1.187	52
S15	3.98	1.185	52
S16	4.02	1.118	52
S17	3.91	1.319	52
S18	***3.98***	***1.248***	***52***
S19	3.6	1.446	52
S20	3.49	1.325	52

S21	3.13	1.52	52
S22	***3.15***	***1.61***	***52***
R1	***3.55***	***1.6***	***52***
R2	***4.15***	***1.116***	***52***
R3	4.11	1.068	52
R4	***4.02***	***1.168***	***52***
R5	4.25	1.175	52
R6	***4.47***	***0.846***	***52***
R7	4.11	1.354	52
R8	4.23	1.086	52
R9	4.13	1.225	52
R10	4.17	0.802	52
R11	4.36	0.901	52

*Bold Italic items not selected

*Significant level of 't'-value at 0.05 & 0.01

Table 3: Correlation between Each Dimension score and overall Score

S. No.	Dimensions	'r' values
A	Home-based involvement	0.320
B	School-based involvement	0.300
C	Responsibilities for learning outcomes	0.554

Table 4: t-values for each dimension of the Parental Involvement

Rejected Items	Group	N	Mean	Std.D	Df	t-value
H1	Upper	27	4.59	.501	52	7.06
	Lower	27	4.37	1.115	52	
H2	Upper	27	3.89	1.739	52	3.74
	Lower	27	3.78	1.251	52	
H5	Upper	27	3.59	1.118	52	3.93
	Lower	27	2.56	1.476	52	
H7	Upper	27	4.19	.786	52	4.24
	Lower	27	3.15	1.486	52	
H10	Upper	27	3.96	1.372	52	1.44
	Lower	27	2.22	1.577	52	
H12	Upper	27	3.74	1.701	52	1.71
	Lower	27	3.93	1.238	52	
H13	Upper	27	4.44	.506	52	9.12
	Lower	27	4.00	1.387	52	
H14	Upper	27	4.81	.396	52	1.23
	Lower	27	4.07	1.328	52	
H15	Upper	27	4.48	.580	52	1.2
	Lower	27	4.07	1.439	52	
H19	Upper	27	4.15	1.027	52	1.05
	Lower	27	3.11	1.577	52	
H22	Upper	27	4.04	1.344	52	4.46
	Lower	27	3.59	1.623	52	

H23	Upper	27	4.41	.501	52	9.17
	Lower	27	2.55	1.578	52	
S1	Upper	27	4.07	1.269	52	0.000033
	Lower	27	3.18	1.588	52	
S6	Upper	27	3.78	1.188	52	0.0001
	Lower	27	3.75	1.456	52	
S7	Upper	27	4.59	.501	52	0.000096
	Lower	27	3.79	1.572	52	
S8	Upper	27	4.33	1.330	52	0.0002
	Lower	27	3.36	1.638	52	
S10	Upper	27	4.33	1.000	52	0.00075
	Lower	27	3.07	1.804	52	
S12	Upper	27	3.74	1.375	52	0.00318
	Lower	27	2.64	1.592	52	
S18	Upper	27	4.37	.839	52	0.00548
	Lower	27	3.46	1.478	52	
S20	Upper	27	3.78	1.32	52	0.01695
	Lower	27	2.66	1.517	52	
S22	Upper	27	3.74	1.403	52	0.02896
	Lower	27	3.18	1.634	52	
R1	Upper	27	4.04	1.372	52	0.02767
	Lower	27	3.18	1.701	52	
R2	Upper	27	4.56	.506	52	0.02559
	Lower	27	3.82	1.389	52	
R4	Upper	27	4.19	1.001	52	0.14195
	Lower	27	3.93	1.303	52	
R6	Upper	27	4.85	.362	52	0.00332
	Lower	27	4.11	.994	52	

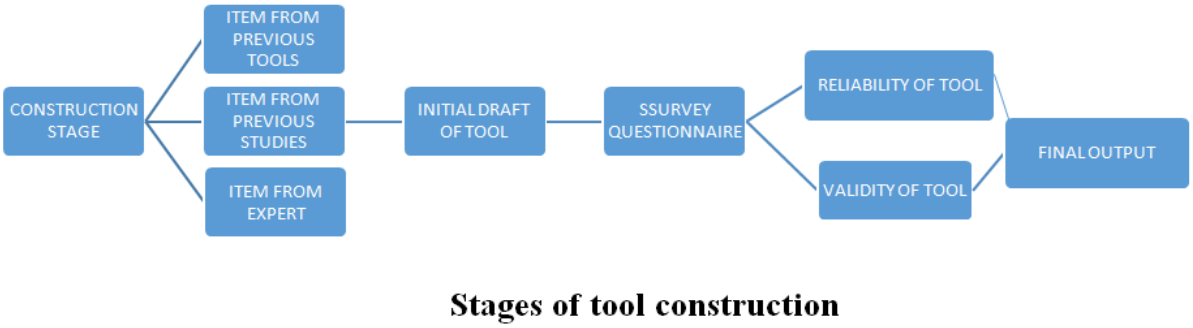
**Level of Significant at 0.05 & 0.01.

Table 5: Cronbach alpha)

S. No.	Dimensions	Alpha
A	Home-based involvement	0.917
B	School-based involvement	0.9
C	Responsibilities for learning outcomes	0.923

Table 6: Item Presentation in the finalizeddraft of Parental Involvement scale

S. No.	Dimensions	Items
A	Home-based involvement	3,4,6,8,9,11,16,17,18,20,21,24,25
B	School-based involvement	27,28,29,30,34,36,38,39,40,41,42,44,46
C	Responsibilities for learning outcomes	50,52,54,55,56,57,58



Stages of tool construction

Figure 1: shows a graphical illustration of the stages of tool construction

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RESEARCH ARTICLE

A Study on Training Need Assessment of Coffee Growers in Kodaikanal Block of Dindigul District

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ABSTRACT

The present case study was conducted in Kodaikanal block of Dindigul district to know the socio-economic profile of the coffee growers, to identify the training need areas of coffee growers and to identify the relationship between the profiles of coffee growers with their training needs. The respondents were selected based on random sampling method. The total sampling was 120 from the four villages; 30 from each village. With respect to the area of training the respondents were collect in an interview schedule on three-point scale like very important, important and not important. The data was analysed by assigning three, two and one score respectively. The responses were coded, tabulated and subjected to descriptive statistical analysis comprising percentage analysis and correlation coefficient was used to identify the relationship. The result reveals that majority (58.33 per cent) of the coffee growers belong to middle age with better socio-economic condition. Majority of the coffee growers had expressed their need for training on recommended coffee varieties, disease management and intercropping.

Keywords: Coffee growers, training needs

INTRODUCTION

Training plays a vital role for capacity building in farming community. The skills required by a coffee cultivator need to be supplemented with additional skills in order to increase the yield of coffee beans. Training is a source of information, advice and influence in the decision making process. Training helps in enhancement of knowledge, improvement of skill, interaction with experts and builds confidence. Keeping this in view, the present case study was conducted to know the training needs with following objectives that include to know the socio- economic profile

of the coffee growers, to identify the training need areas of coffee growers and to study the relationship between the profiles of coffee growers with its training need.

METHODOLOGY

The study was conducted in Kodaikanal block of Dindigul district of Tamil Nadu, since the district had highest, production and productivity. The respondent was selected on random sampling technique of coffee growers were selected for the study. The total sampling was 120 from the four villages (30 from each village).With respect to the area of training the respondents were collect in an interview schedule on three-point scale like very important, important and not important. The data was analysed by assigning three, two and one score respectively. Mean score of each training area were calculated by using the formula sum of the terms divided by number of terms. The responses were coded, tabulated and subjected to descriptive statistical analysis comprising percentage analysis and correlation coefficient was used to identify the relationship.

RESULTS AND DISCUSSION

Distribution of respondents based on their profile characteristics, majority of the coffee farmers were middle aged (58.33 per cent) with primary school education (54.16 per cent) belongs to BC category (53.33 per cent), high experience (58.33 per cent), medium social participation (56.66 per cent), medium contact with extension agency (58.33 per cent), medium annual income (54.16 per cent), large land holding (68.33 per cent), medium scientific orientation (54.16 per cent) and medium market orientation (53.33 per cent) respectively. This finding is in line with findings of Patil (2011). The study on training needs (Table 3) revealed that (61.66 per cent) of the respondents were under medium training needs category, (20.00 per cent) had low training need whereas rest of (18.34 per cent) had high training need. It can be concluded that majority of the coffee growers (61.66 per cent) fell under medium group, while (20.00 per cent) and (18.34 per cent) of the coffee growers were categorized under high and low groups of training needs, respectively. This finding is in line with findings of Bhise et al (2016). The Table 4 shows that among ten characteristics studied, six characteristics namely, education, social participation, extension contact, scientific orientation and market orientation were found to have negatively and significantly correlation with training needs at 0.01 level of probability. Whereas, age, experience in coffee cultivation, annual income and land holding were correlated negatively and significantly at 0.05 level of probability. Further, caste did not show any relationship with training needs. This finding is in line with findings of Hashemi (2012).

CONCLUSION

It can be concluded from the study that majority of the coffee growers had expressed their need for training on recommended coffee varieties, disease management and intercropping. The analysis of correlation of selected characteristics like age, education, and experience in coffee cultivation, social participation, extension contact, annual income, land holding, scientific orientation and market orientation were found negatively and significantly towards their training needs.

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Table 1: Distribution of respondents according to their profile characteristics

(n= 120)

S.No	Personality traits	Categories	No of respondents	Percentage
1	**Age**	Young	10	08.33
		Adult	70	58.33
		Old	40	33.33
2	**Education**	Primary	65	54.16
		Higher secondary	40	33.33
		College	15	12.50
3	**Experience**	Less	10	08.33
		Medium	40	33.33
		High	70	58.33
4	**Caste**	SC	25	20.83
		BC	64	53.33
		MBC	31	25.83
5	**Social participation**	Low	30	25.00
		Medium	68	56.66
		High	22	18.33
6	**Extension contact**	Low	30	25.00
		Medium	70	58.33
		High	20	16.66
7	**Annual income**	Low	30	25.00
		Medium	65	54.16
		High	25	20.83
8	**Land holding**	Small	9	07.50
		Marginal	29	24.16
		Large	82	68.33
9	**Scientific orientation**	Low	35	29.16
		Medium	65	54.16
		High	20	16.66
10	**Market orientation**	Low	36	30.00
		Medium	64	53.33
		High	20	16.66

Table 2: Distribution of the coffee growers according to their training needs

(n= 120)

S.No	Areas	Very important	Important	Not important
1	Land preparation	48 (40.00%)	53(44.50%)	19 (15.50%)
2	Recommended coffee variety	57 (47.50%)	42 (35.00%)	21 (17.50%)
3	Nursery management	50 (41.67%)	49 (40.84 %)	21 (17.50 %)
4	Manure and fertilizer	47 (39.16%)	51 (42.50%)	22 (18.33%)
5	Intercropping	56 (46.66%)	44 (36.66%)	20 (16.66%)
6	Water management	45 (37.50%)	52 (43.33%)	23 (19.16%)
7	Pest management	53 (44.50%)	48 (40.00%)	19 (15.50%)
8	Disease management	58 (48.33%)	41 (34.16%)	21 (17.50%)

9	Harvesting time	50 (41.67%)	49 (40.84 %)	21 (17.50%)
10	Marketing	44 (36.66%)	53 (44.50%)	23 (19.16%)
11	Post-harvest techniques	46 (38.33%)	50 (41.67%)	24 (20.00%)

Table 3: Over all distribution of the coffee growers according to their training needs

(n= 120)

S.No	Training needs	Frequency	Percentage
1	High	22	18.34
2	Low	74	61.66
3	Medium	24	20.00
Total		**120**	**100.00**

Table 4: Correlation between profiles and training need of the coffee

(n= 120)

S.No	Independent Variable	Correlation Coefficient r value
1	Age	-0.2071*
2	Education	-0.7455*
3	Experience	-0.1817*
4	Caste	0.0831NS
5	Social participation	-0.7023**
6	Extension contact	-0.2716**
7	Annual income	-0.1792*
8	Land holding	-0.1777*
9	Scientific orientation	-0.6764**
10	Market orientation	-0.7034**

** - Significant at 1 per cent level

* - Significant at 5 per cent level

NS - Non- Significant

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RESEARCH ARTICLE

Impact of Virtual Reality on Improving Upper Limb Function in Stroke Patients: A Case Series Study

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ABSTRACT

Recovery of precise hand functions after stroke is poor. Virtual reality therapy facilitates feedback mechanism enhancing easy accommodation by promoting motor learning based functional activities such as gripping. To find out the effect of virtual reality therapy in improving upper limb function post stroke. Five patients diagnosed with left middle cerebral artery stroke with six months duration were treated with assistive virtual reality exercises for six weeks, five sessions each week, each session lasting for forty-five minutes. Patient was again assessed with fugl meyer scale to identify the changes. Student t test was used. Significant improvement in upper limb function after virtual reality therapy were observed. Virtual reality-based therapy added with is more effective for improving upper limb function in individuals with chronic stroke than using the conventional method alone.

Keywords: virtual reality, chronic stroke, upper limb, medial cerebellar artery (MCA)

INTRODUCTION

Stroke is a pronounced disturbance that leads to absurd impact on the function and quality of life. Almost 55% to 75% of the survivors endure motor deficits affecting motor control, fine motor skills and dual task coordination abilities. Virtual reality, as an integrative approach with the conventional mode fastens up the recovery[1]. The use of multisensory simulated environment and real time feedback on performance encourages the patient to concentrate

their attention completely on the task, thus improving motivation and treatment compliance, while recovering from stroke. The outlook of a virtual reality-based exercise program (VREP) is considered to work upon the paralyzed upper limb component of the patients focussing to promote better daily activities. The integration of virtual reality is that, the stroke patient can think of it as an interesting perceptive work, better than considering as a treatment. This improves the focus and cognition aspects of the patients, thereby working upon the task-oriented activities[2].

METHODOLOGY

Five patients, diagnosed with left MCA stroke with six-months duration (brunnstrom stage of recovery 3, with a spasticity grade 2 on modified ashworth's scale)as identified by the neurophysician were selected to fulfill the purpose of this research. Extension at wrist was 15º and 10º for fingers from the full flexion, making it easy to involve in performing utility activities. Consent was sought and the patients were assessed with fugl meyer scale, to understand the difference in the levels of improvement, before and after administering virtual reality therapy, along with the conventional protocol. The patient's cooperated well all the precise instructions. The study was conducted in the outpatient department of a private physiotherapy college in Kerala. The outcome of this experimental research measure was based on the fugl meyer scale. The treatment was administered in person for about 45 minutes each day, 5 session per week for 6 weeks duration, between the commencement and after completion of treatment were analysed.

Data analysis
Student t test was used – pretest and posttest method

RESULT

The posttest value of fugl meyer scale showed significant improvement in upper limb function of stroke patient.

DISCUSSION

This research aimed to know the prospects of virtual reality in aiding the functional recovery of the affected upper limb post stroke. Precise activities, encourages the participants to re- learn, by facilitating sensory motor actions that aid control of muscle contraction, channelled neuro muscular activity, improved range of motion, using the affected upper limb. Precision and prehension are essential components that ensure lost function being streamlined. As a source of equalizing the concentrative and concretive ability of the patient, VREP has been designed as a tool of entertainment, that grabs the attention into participation towards the rehabilitative program. The study by Antonio Vinicius et al., proved that virtual reality-based programs are accelerative to facilitate recovery that involves the motor component in chronic stroke [3]. Jang et al, documented that improvement could be observed by involving interactive rehabilitation and exercise system ideologies of virtual reality that aided reorganization of cortical system and task-oriented arm function in post stroke [4]. The strength of the muscle which is essential to execute any work improved using VR based activities as reported Reneh et al., facilitating better ADL[5]. The present research work, involving the advocacy of fugl meyer scale showed significant changes, with observable increments in assessment (as seen in the table and graph), when administered virtual reality on five chronic stroke survivors. Flexibility and accommodation in movement, reduced pain, improved function process was also documented. Stroke rehabilitation on long term survivors, is aided-up by virtual reality training, while the differential component that fosters preciseness in motor actions and sensory implications can be observed. The vast continuing exploration on virtual reality based rehabilitative programs, reduced cost of treatment and improved ADL can be witnessed in any monitored set-up, as the regular activities most commonly demand extra efforts and time to be performed in hospital[6].

CONCLUSION

This study helped to understand that virtual reality added with conventional module helps to enhance the functional attributes of the upper limb in patients affected with stroke, in a short span of time.

Recommendation: The study can be done on Anterior cerebral artery stroke and Posterior cerebral artery stroke.
Conflict of interest: None
Source of fund : Self

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Table 1: Represents the difference in the pre and post-test values of fugl meyer scale

Fugl Meyer Scale		
Sl. No	Pretest score	Posttest score
1.	34	40
2.	33	36
3.	36	40
4.	35	39
5.	35	40

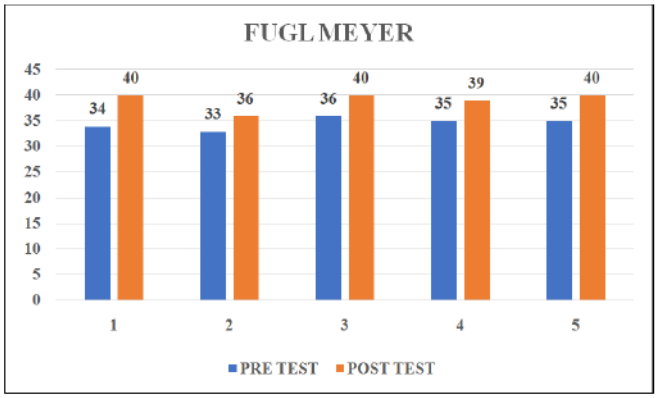

Fig. 1: Graph represents the change in pre and post-test analysis

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RESEARCH ARTICLE

Correlation Coefficient for Cubic Picture Fuzzy Soft Matrices

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ABSTRACT

In this article, we present the notion of correlation coefficient using cubic picture fuzzy soft matrices. The correlation coefficient is used to measure the relationship between two variables. The formation of cubic picture fuzzy set is a combination of interval-value and fuzzy membership values for every positive, neutral and negative value. Also, we study some of its theoretical approaches with suitable examples. Finally, we discuss an algorithm and a case study for the selection of the best cashews available in the market.

Keywords: picturefuzzy soft set, cubic softset, correlation, correlation coefficient.

INTRODUCTION

Zadeh [10] introduced the notion of fuzzy set (FS) and discussed the notion of linguistic variables and their application to reasoning. Atanassov [2] presented the notion of intuitionistic FS (IFS). Molodtsov [9] presented the theory of soft set (SS) to deal with uncertainty. Maji. et al. [8] defined the concept of fuzzy SS (FSS) by combining FS with SS. Jun.et al. [7] proposed the concept of cubic set (CS) by combining interval-valued FS and FS. Cuong [5] approaches the idea of Picture FS (PFS) with a condition that the membership values of truth, indeterminacy, and falsity cannot be greater than one. Cagman and Enginoglu [3] presented the theory of fuzzy soft matrix (FSM). Dogra and Pal [6] extended the notion of PFS to Picture fuzzy matrix (PFM) and discussed some of its properties. Ashraf et al., [1] proposed cubic picture fuzzy sets and established some of its properties. Chinnadurai and Madhanraj [4] have discussed the concept of cubic picture fuzzy soft matrix (CPFSM), which extends CPFSS.

INFORMATIONAL ENERGY FOR CPFSM

Let

$P_C^M = \{(s_i)_j, < [\underline{\alpha}_p(s_i)_j, \overline{\alpha}_p(s_i)_j], \alpha_p(s_i)_j >, < [\underline{\beta}_p(s_i)_j, \overline{\beta}_p(s_i)_j], \beta_p(s_i)_j >, < [\underline{\gamma}_p(s_i)_j, \overline{\gamma}_p(s_i)_j], \gamma_p(s_i)_j >, (s_i)_j \in S, i, j = 1.2, \dots, m\}$

, be the CPFSM then the informational energy,

$$E_I(P_C^M) = \sum_{i,j=1}^{m} \left(\left\{ < \left[2\left(\underline{\alpha_p}^2(s_i)_j\right) + 2\left(\overline{\alpha_p^2}(s_i)_j\right)\right] + 2\left(\alpha_p{}^2(s_i)_j\right) + \left\{ < \left[\underline{\beta_p}^2(s_i)_j + \overline{\beta_p^2}(s_i)_j\right] + \beta_p{}^2(s_i)_j > \right\} + \left\{ < \left[\underline{\gamma_p}^2(s_i)_j + \overline{\gamma_p^2}(s_i)_j\right] + \gamma_p{}^2(s_i)_j > \right\} \right\} \right)$$

CORRELATION FOR CPFSMs

For two CPFSMs P_C^M and Q_C^M ,

$P_C^M = \{(s_i)_j, < [\underline{\alpha}_p(s_i)_j, \overline{\alpha}_p(s_i)_j], \alpha_p(s_i)_j >, < [\underline{\beta}_p(s_i)_j, \overline{\beta}_p(s_i)_j], \beta_p(s_i)_j >, < [\underline{\gamma}_p(s_i)_j, \overline{\gamma}_p(s_i)_j], \gamma_p(s_i)_j >, (s_i)_j \in S, i, j = 1.2, \dots, m\}$

and

$Q_C^M = \{x_{ij}, < [\underline{\alpha}_Q(s_i)_j, \overline{\alpha}_Q(s_i)_j], \alpha_Q(s_i)_j >, < [\underline{\beta}_Q(s_i)_j, \overline{\beta}_Q(s_i)_j], \beta_Q(s_i)_j >, < [\underline{\gamma}_Q(s_i)_j, \overline{\gamma}_Q(s_i)_j], \gamma_Q(s_i)_j >, (s_i)_j \in S, i, j = 1.2, \dots, m\}$

respectively, then their correlation is defined by

$$C_R(P_C^M, Q_C^M) = \sum_{i,j=1}^{m} \left\{ 2\{\underline{\alpha}_p(s_i)_j . \underline{\alpha}_Q(s_i)_j\}^2 + 2\{\overline{\alpha}_p(s_i)_j . \overline{\alpha}_Q(s_i)_j\}^2 + 2\{\alpha_p(s_i)_j . \alpha_Q(s_i)_j\}^2 + \left(\underline{\beta}_p(s_i)_j . \underline{\beta}_Q(s_i)_j + \overline{\beta}_p(s_i)_j . \overline{\beta}_Q(s_i)_j + \beta_p(s_i)_j . \beta_Q(s_i)_j\right) + \left(\underline{\gamma}_p(s_i)_j . \underline{\gamma}_Q(s_i)_j + \overline{\gamma}_p(s_i)_j . \overline{\gamma}_Q(s_i)_j + \gamma_p(s_i)_j . \gamma_Q(s_i)_j\right) \right\}$$

For P_C^M , $Q_C^M \in$ CPFSMs, then the above equation satisfies the following condition, *i.e.*, $C_R(P_C^M, Q_C^M) = C_R(Q_C^M, P_C^M)$.

CORRELATION COEFFICIENT FOR CPFSMs

Let P_C^M and Q_C^M be two CPFSMs,

$P_C^M = \{(s_i)_j, < [\underline{\alpha}_p(s_i)_j, \overline{\alpha}_p(s_i)_j], \alpha_p(s_i)_j >, < [\underline{\beta}_p(s_i)_j, \overline{\beta}_p(s_i)_j], \beta_p(s_i)_j >, < [\underline{\gamma}_p(s_i)_j, \overline{\gamma}_p(s_i)_j], \gamma_p(s_i)_j >, (s_i)_j \in S, i, j = 1.2, \dots, m\}$

and

$Q_C^M = \{x_{ij}, < [\underline{\alpha}_Q(s_i)_j, \overline{\alpha}_Q(s_i)_j], \alpha_Q(s_i)_j >, < [\underline{\beta}_Q(s_i)_j, \overline{\beta}_Q(s_i)_j], \beta_Q(s_i)_j >, < [\underline{\gamma}_Q(s_i)_j, \overline{\gamma}_Q(s_i)_j], \gamma_Q(s_i)_j >, (s_i)_j \in S, i, j = 1.2, \dots, m\}$

respectively. Then the correlation coefficient between P_C^M and Q_C^M is defined by

$$C_{CO}(P_C^M, Q_C^M) = \frac{C_R(P_C^M, Q_C^M)}{\left(E_I(P_C^M)\right)^{\frac{1}{2}} \left(E_I(Q_C^M)\right)^{\frac{1}{2}}} \Rightarrow \sum_{i,j=1}^{m} \left\{ 2\{\underline{\alpha}_p(s_i)_j . \underline{\alpha}_Q(s_i)_j\}^2 + 2\{\overline{\alpha}_p(s_i)_j . \overline{\alpha}_Q(s_i)_j\}^2 + 2\{\alpha_p(s_i)_j . \alpha_Q(s_i)_j\}^2 + \left(\underline{\beta}_p(s_i)_j . \underline{\beta}_Q(s_i)_j + \overline{\beta}_p(s_i)_j . \overline{\beta}_Q(s_i)_j + \beta_p(s_i)_j . \beta_Q(s_i)_j\right) + \left(\underline{\gamma}_p(s_i)_j . \underline{\gamma}_Q(s_i)_j + \overline{\gamma}_p(s_i)_j . \overline{\gamma}_Q(s_i)_j + \gamma_p(s_i)_j . \gamma_Q(s_i)_j\right) \right\} /$$

$$\left(\sum_{i,j=1}^{m}\left(\left(<\left[2\left(\underline{\alpha_P}^2(\delta_i)_j\right)+2\left(\overline{\alpha_P}^2(\delta_i)_j\right)\right]+2\left(\alpha_P{}^2(\delta_i)_j\right)>+<\left[\underline{\beta_P}^2(\delta_i)_j+\overline{\beta_P}^2(\delta_i)_j\right]+\beta_P{}^2(\delta_i)_j>+<\left[\underline{\gamma_P}^2(\delta_i)_j+\overline{\gamma_P}^2(\delta_i)_j\right]+\gamma_P{}^2(\delta_i)_j>\right)\right)\right)^{\frac{1}{2}}$$

.

$$\left(\sum_{i,j=1}^{m}\left(\left(<\left[2\left(\underline{\alpha_Q}^2(\delta_i)_j\right)+2\left(\overline{\alpha_Q}^2(\delta_i)_j\right)\right]+2\left(\alpha_Q{}^2(\delta_i)_j\right)>+<\left[\underline{\beta_Q}^2(\delta_i)_j+\overline{\beta_Q}^2(\delta_i)_j\right]+\beta_Q{}^2(\delta_i)_j>+<\left[\underline{\gamma_Q}^2(\delta_i)_j+\overline{\gamma_Q}^2(\delta_i)_j\right]+\gamma_Q{}^2(\delta_i)_j>\right)\right)\right)^{\frac{1}{2}}$$

EXAMPLE

Let us consider,

$P_C^M = (<(2([0.16,0.21],0.23),<[0.21,0.30],0.22>,<[0.32,0.34],0.33>)$

$\Rightarrow (<[0.32,0.42],0.46>,<[0.21,0.30],0.22>,<[0.32,0.34],0.33>)$

$Q_C^M = (<(2([0.25,0.35],0.09>),<[0.23,0.32],0.26>,<[0.10,0.17],0.28>)$

$\Rightarrow (<[0.50,0.70],0.18>,<[0.23,0.32],0.26>,<[0.10,0.17],0.28>)$

By using definition 3.1, we can calculate the informational energy of $\mathbb{P}_C^M$ and Q_C^M as follows:

$E_I(\mathbb{P}_C^M) = (<[0.1024+0.1764]+0.2116>+<[0.0441+0.0900]+0.0484>+<[0.1024+0.1156]+0.1089>)$

$\Rightarrow (<0.4904>+<0.1825>+<0.3269>) = 0.9998$

$\left(E_I(P_C^M)\right)^{\frac{1}{2}} = 0.9998$

$E_I(Q_C^M) = (<[0.2500+0.4900]+0.0324>+<[0.0529+0.1936]+0.0676>+<[0.0100+0.0289]+0.0784>)$

$\Rightarrow (<0.7724>+<0.3141>+<0.1173>) = 1.2038 \left(E_I(Q_C^M)\right)^{\frac{1}{2}} = 1.0971$

$C_R(P_C^M, Q_C^M)=$

$(<[(\{2\{(0.16).(0.25)\}^2\})+(\{2\{(0.21).(0.35)\}^2\})]+(\{2\{(0.23).(0.09)\}\}^2)>+<[(0.21).(0.23)+(0.30).(0.32)]+(0.22).(0.26)>+<[(0.32).(0.10)+(0.34).(0.17)]+(0.33).(0.28)>)$

$\Rightarrow (<0.0148>+<0.2015>+<0.7024>) = 0.9187$

By using definition 3.4, we can compute the correlation coefficient between $\mathbb{P}_C^M$ and Q_C^M as follows:

$$\Rightarrow C_{CO}(\mathbb{P}_C^M, Q_C^M) = \frac{C_R(P_C^M, Q_C^M)}{\left(E_I(P_C^M)\right)^{\frac{1}{2}}\left(E_I(Q_C^M)\right)^{\frac{1}{2}}}$$

$$\Rightarrow \frac{0.9187}{(0.9998)^{\frac{1}{2}}.(1.2038)^{\frac{1}{2}}}$$

$$\Rightarrow \frac{0.9187}{(0.9998).(1.0971)} = \frac{0.9187}{1.0968} = 0.84$$

Application of CPFSMS in Selecting the Best Cashews

In this section, we use the concepts of informational energy, correlation coefficient, and total score of the correlation coefficient for CPFSMs for selecting the best cashews available in the market. Also, we provide an application of CPFSMs to solve multi criteria decision-making problems. We develop an algorithm and illustrate the working of the algorithm with a suitable example.

Total score for CPFSMS

The total score for the correlation coefficient for CPFSMs helps to transform the elements of cubic picture fuzzy matrices into a real number to bring out the significance of these membership values. Each element of a cubic picture fuzzy matrix is a combination of three closed subinterval of [0,1] and a real number of [0,1]. It is necessary to

integrate the correlation coefficient value for two matrices into a single real number to arrive at a numerical value that can be viewed as a measure of that property.

$C_{CO}(P_C^M, Q_C^M) = \frac{C_R(P_C^M, Q_C^M)}{\left(E_I(P_C^M)\right)^{\frac{1}{2}} \left(E_I(Q_C^M)\right)^{\frac{1}{2}}}$ Then the total score for the correlation coefficient for CPFSMs is given below:

$V(C_{CO}(P_C^M, Q_C^M)) = \frac{1}{4}\sum(C_{CO}(P_C^M, Q_C^M))$

Statement of the Problem

Let $\mathbb{U} = \{c_1, c_2, c_3, c_4\}$ be a set of cashew nuts taken into consideration. Assume that the agricultural experts select the cashews based on plant food, bio-pesticides, natural resources and fertility parameters. Let $\mathbb{H} = \{h_1, h_2, h_3, h_4\}$ be a set of parameters, where $h_1 = \text{color of the nuts}, h_2 = \text{size of the nuts}, h_3 = \text{longer expiration date}, h_4 = \text{kernal shapes}$ Now, the problem is to present the values in CPFSMs for selection of best cashews.

Algorithm

The algorithm for ranking the cashew nuts based on the correlation coefficient of CPFSMs is given below:

Step 1: Identify the cashew nuts and the list of parameters.

Step 2: Assign a cubic picture fuzzy value for each parameter.

Step 3: Construct the CPFSMs for each of the experts.

Step 4: Calculate the correlation coefficient by using informational energy and correlation values.

Step 5: Evaluate the total value $V(C_{CO}(P_C^M, Q_C^M))$ for each cashew nut.

Step 6: Order the $V(C_{CO}(P_C^M, Q_C^M))$ values,then choose the one with highest total value as the bestcashew nut.

CASESTUDY

The agricultural experts are in the process of selecting the cashew nuts.

1. Let $\mathbb{U} = \{c_1, c_2, c_3, c_4\}$ be a set of cashew nuts.
2. Let $\mathbb{H} = \{h_1, h_2, h_3, h_4\}$ be a set of parameters for selection of best cashew nuts.
3. Form CPFSMs as

$$P_C^M = \begin{bmatrix} <[0.21,0.34],0.24>,<[0.11,0.25],0.15>,<[0.25,0.31],0.24> \\ <[0.27,0.37],0.11>,<[0.25,0.46],0.28>,<[0.12,0.19],0.30> \\ <[0.16,0.26],0.20>,<[0.15,0.37],0.38>,<[0.22,0.40],0.41> \\ <[0.25,0.29],0.27>,<[0.29,0.32],0.30>,<[0.19,0.33],0.31> \end{bmatrix}$$

$$\begin{bmatrix} <[0.20,0.30],0.25>,<[0.31,0.41],0.40>,<[0.16,0.27],0.28> \\ <[0.17,0.37],0.18>,<[0.21,0.27],0.25>,<[0.26,0.36],0.45> \\ <[0.18,0.20],0.19>,<[0.35,0.41],0.34>,<[0.27,0.38],0.20> \\ <[0.16,0.21],0.23>,<[0.21,0.30],0.22>,<[0.32,0.34],0.33> \end{bmatrix}$$

$$\begin{bmatrix} <[0.26,0.35],0.16>,<[0.19,0.25],0.27>,<[0.16,0.32],0.42> \\ <[0.17,0.27],0.21>,<[0.16,0.38],0.37>,<[0.23,0.31],0.40> \\ <[0.19,0.21],0.25>,<[0.34,0.39],0.30>,<[0.28,0.37],0.22> \\ <[0.15,0.20],0.25>,<[0.20,0.29],0.23>,<[0.31,0.33],0.40> \end{bmatrix}$$

$$\begin{bmatrix} <[0.24,0.28],0.25>,<[0.26,0.30],0.32>,<[0.18,0.23],0.33> \\ <[0.21,0.31],0.20>,<[0.32,0.40],0.39>,<[0.19,0.25],0.29> \\ <[0.26,0.35],0.12>,<[0.24,0.45],0.29>,<[0.11,0.20],0.40> \\ <[0.16,0.17],0.21>,<[0.37,0.43],0.36>,<[0.29,0.40],0.22> \end{bmatrix}$$

$$Q_C^M = \begin{bmatrix} <[0.22,0.32],0.21>,<[0.33,0.41],0.40>,<[0.20,0.26],0.30> \\ <[0.23,0.36],0.26>,<[0.13,0.27],0.17>,<[0.27,0.33],0.26> \\ <[0.17,0.22],0.27>,<[0.22,0.31],0.25>,<[0.33,0.35],0.42> \\ <[0.21,0.31],0.26>,<[0.32,0.42],0.41>,<[0.17,0.28],0.29> \end{bmatrix}$$

$$\begin{matrix}
<[0.18,0.20],0.24>,<[0.33,0.37],0.29>,<[0.27,0.36],0.21> \\
<[0.19,0.21],0.20>,<[0.36,0.42],0.35>,<[0.28,0.39],0.19> \\
<[0.18,0.28],0.22>,<[0.17,0.39],0.40>,<[0.24,0.30],0.38> \\
<[0.25,0.35],0.09>,<[0.23,0.44],0.26>,<[0.10,0.17],0.28> \\
<[0.19,0.29],0.18>,<[0.30,0.38],0.37>,<[0.17,0.23],0.31> \\
<[0.26,0.30],0.28>,<[0.30,0.33],0.31>,<[0.20,0.34],0.32> \\
<[0.15,0.25],0.19>,<[0.14,0.36],0.35>,<[0.21,0.29],0.38> \\
<[0.25,0.34],0.11>,<[0.23,0.44],0.28>,<[0.10,0.19],0.28>
\end{matrix}$$

$$\begin{bmatrix}
<[0.15,0.18],0.7>,<[0.33,0.39],0.32>,<[0.25,0.35],0.18> \\
<[0.18,0.38],0.19>,<[0.22,0.27],0.26>,<[0.27,0.35],0.45> \\
<[0.23,0.32],0.22>,<[0.34,0.40],0.41>,<[0.21,0.23],0.32> \\
<[0.19,0.29],0.23>,<[0.18,0.40],0.39>,<[0.25,0.31],0.38>
\end{bmatrix}$$

(4) Using Definition 3.1, the computation of informational energy is as below

$$E_I(P_C^M) = \begin{bmatrix}
([0.1764+0.4624]+0.3364)+([0.0121+0.0625]+0.0225)+([0.0625+0.0961]+0.0576) \\
([0.2916+0.5476]+0.0484)+([0.0625+0.2116]+0.0784)+([0.0144+0.0361]+0.0900) \\
([0.1024+0.2704]+0.1600)+([0.0225+0.1369]+0.1444)+([0.0484+0.1600]+0.1681) \\
([0.2500+0.3364]+0.2916)+([0.0841+0.1024]+0.0900)+([0.0361+0.1089]+0.0961)
\end{bmatrix}$$

$$\begin{matrix}
([0.1600+0.3600]+0.2500)+([0.0961+0.1681]+0.1600)+([0.0256+0.0729]+0.0784) \\
([0.1156+0.1369]+0.1296)+([0.0441+0.0729]+0.0625)+([0.0676+0.1296]+0.2025) \\
([0.1296+0.1600]+0.1444)+([0.1225+0.1681]+0.1156)+([0.0729+0.1444]+0.0400) \\
([0.1024+0.1764]+0.2116)+([0.0441+0.0900]+0.0484)+([0.1024+0.1156]+0.1089)
\end{matrix}$$

$$\begin{matrix}
([0.2704+0.1225]+0.1024)+([0.0361+0.0625]+0.0729)+([0.0256+0.1024]+0.1764) \\
([0.1156+0.2916]+0.1764)+([0.0256+0.1444]+0.1369)+([0.0529+0.0961]+0.1600) \\
([0.1444+0.1764]+0.2500)+([0.1156+0.1521]+0.0900)+([0.0784+0.1369]+0.0484) \\
([0.0900+0.1600]+0.2500)+([0.0400+0.0841]+0.0529)+([0.0961+0.1089]+0.1600)
\end{matrix}$$

$$\begin{bmatrix}
([0.2304+0.3136]+0.2500)+([0.0676+0.0900]+0.1024)+([0.0324+0.0529]+0.1089) \\
([0.1764+0.3844]+0.1600)+([0.1024+0.1600]+0.1521)+([0.0361+0.0625]+0.0841) \\
([0.2704+0.4900]+0.0576)+([0.0576+0.2025]+0.0841)+([0.0121+0.0400]+0.1600) \\
([0.1024+0.1156]+0.1764)+([0.1369+0.1849]+0.1296)+([0.0841+0.1600]+0.0484)
\end{bmatrix}$$

$$\Rightarrow \begin{bmatrix}
<1.2885><1.2711><0.9712><1.2482> \\
<1.3806><0.9613><1.1995><1.3180> \\
<1.2131><1.0975><1.1922><1.3743> \\
<1.3956><0.9998><1.0420><1.1383>
\end{bmatrix}$$

$$\left(E_I(P_C^M)\right)^{\frac{1}{2}} = \begin{bmatrix}
<1.1351><1.1274><0.9854><1.1172> \\
<1.1749><0.9804><1.0952><1.1480> \\
<1.1014><1.0476><1.0918><1.1723> \\
<1.1813><0.9998><1.0207><1.0669>
\end{bmatrix}$$

$$E_I(Q_C^M) = \begin{bmatrix}
([0.1936+0.4096]+0.1764)+([0.1089+0.1681]+0.1600)+([0.0400+0.0676]+0.0900) \\
([0.2116+0.5184]+0.2704)+([0.0169+0.0729]+0.0289)+([0.0729+0.1089]+0.0676) \\
([0.1156+0.1936]+0.2916)+([0.0484+0.0961]+0.0625)+([0.1089+0.1225]+0.1764) \\
([0.1764+0.3844]+0.2704)+([0.1024+0.1764]+0.1681)+([0.0289+0.0784]+0.0841)
\end{bmatrix}$$

$$([0.1296+0.1600]+0.2304)+([0.1089+0.1369]+0.0841)+([0.0729+0.1296]+0.4410)$$
$$([0.1444+0.1764]+0.1600)+([0.1296+0.1764]+0.1225)+([0.0784+0.1521]+0.0361)$$
$$([0.1296+0.3136]+0.1936)+([0.0289+0.1521]+0.1600)+([0.0576+0.0900]+0.1444)$$
$$([0.2500+0.4900]+0.0324)+([0.0529+0.1936]+0.0676)+([0.0100+0.2890]+0.0784)$$

$$([0.1444+0.3364]+0.1296)+([0.0900+0.1444]+0.1369)+([0.0289+0.0529]+0.0961)$$
$$([0.2704+0.3600]+0.3136)+([0.0900+0.1089]+0.0961)+([0.0400+0.1156]+0.1024)$$
$$([0.0900+0.2500]+0.1444)+([0.0196+0.1296]+0.1225)+([0.0441+0.0841]+0.1444)$$
$$([0.2500+0.4624]+0.0484)+([0.0529+0.1936]+0.0784)+([0.0100+0.0361]+0.0784)$$

$$\begin{bmatrix}([0.0900+0.1296]+0.1156)+([0.1089+0.1521]+0.1024)+([0.0625+0.1225]+0.0324)\\([0.1296+0.5776]+0.1444)+([0.0484+0.0729]+0.0676)+([0.0729+0.1225]+0.2025)\\([0.2116+0.4096]+0.1936)+([0.1156+0.1600]+0.1681)+([0.0441+0.0529]+0.1024)\\([0.1444+0.3364]+0.2116)+([0.0324+0.1600]+0.1521)+([0.0625+0.0961]+0.1444)\end{bmatrix}$$

$$\Rightarrow \begin{bmatrix}<1.4142><1.4934><1.1596><0.9160>\\<1.3685><1.1759><1.4970><1.4384>\\<1.2156><1.2698><1.0287><1.4579>\\<1.4695><1.2038><1.2102><1.3399>\end{bmatrix}$$

$$\left(E_I(Q_C^M)\right)^{\frac{1}{2}} = \begin{bmatrix}<1.1892><1.2220><1.0768><0.9570>\\<1.1698><1.0843><1.2235><1.1993>\\<1.1025><1.1268><1.0142><1.2074>\\<1.2122><1.0971><1.1000><1.1575>\end{bmatrix}$$

$$\left[\left(E_I(P_C^M)\right)^{\frac{1}{2}}.\left(E_I(P_C^M)\right)^{\frac{1}{2}}\right] = \begin{bmatrix}<1.3498><1.3776><1.0610><1.0691>\\<1.3743><1.0630><1.3399><1.3767>\\<1.2142><1.1804><1.1073><1.4154>\\<1.4319><1.0968><1.1227><1.2349>\end{bmatrix}$$

(5) Using Definition 3.2, the correlation matrix is obtained as

$$C_R(P_C^M, Q_C^M) = \begin{bmatrix}([0.0341+0.1893]+0.0593)+([0.0363+0.1025]+0.0600)+([0.0500+0.0806]+0.0720)\\([0.0617+0.2838]+0.0130)+([0.0325+0.1242]+0.0476)+([0.0324+0.0627]+0.0780)\\([0.0118+0.0523]+0.0466)+([0.0330+0.1147]+0.0950)+([0.0726+0.1400]+0.1722)\\([0.0441+0.1293]+0.0788)+([0.0928+0.1344]+0.1230)+([0.0323+0.0924]+0.0899)\end{bmatrix}$$

$$([0.0207+0.0576]+0.5760)+([0.1023+0.1517]+0.1160)+([0.0162+0.0972]+0.0588)$$
$$([0.0166+0.0965]+0.0207)+([0.0756+0.1134]+0.0875)+([0.0728+0.1404]+0.0855)$$
$$([0.0167+0.0501]+0.0279)+([0.0595+0.1599]+0.1360)+([0.0648+0.1140]+0.0760)$$
$$([0.0256+0.0864]+0.0828)+([0.4400+0.1320]+0.0572)+([0.0320+0.0578]+0.0924)$$

$$([0.0390+0.1648]+0.0132)+([0.0570+0.0950]+0.0999)+([0.0272+0.0736]+0.1302)$$
$$([0.0572+0.1866]+0.0847)+([0.0390+0.0891]+0.0527)+([0.0540+0.1122]+0.0832)$$
$$([0.0129+0.0441]+0.0361)+([0.0476+0.1404]+0.1050)+([0.0588+0.1073]+0.0836)$$
$$([0.0441+0.1777]+0.0130)+([0.0736+0.1848]+0.1148)+([0.0170+0.0532]+0.0812)$$

$$\begin{bmatrix}([0.0116+0.0207]+0.1632)+([0.1089+0.1443]+0.0928)+([0.0675+0.1260]+0.0378)\\([0.0187+0.1018]+0.0231)+([0.0729+0.1134]+0.0910)+([0.0756+0.1365]+0.0855)\\([0.0274+0.1284]+0.0374)+([0.0578+0.1560]+0.1640)+([0.0504+0.0690]+0.1216)\\([0.0361+0.1648]+0.0068)+([0.0414+0.1760]+0.1014)+([0.0250+0.0527]+0.1064)\end{bmatrix}$$

$$\Rightarrow \begin{bmatrix}<0.6841><0.6781><0.6999><0.7728>\\<0.7359><0.7090><0.7587><0.7248>\\<0.7382><0.7049><0.6358><0.8120>\\<0.8170><0.9169><0.7594><0.7106>\end{bmatrix}$$

(6) Using Definition 3.3, the correlation coefficients are calculated as

$$C_{CO}(P_C^M, Q_C^M) = \begin{bmatrix} <0.5068><0.4922><0.6596><0.7228> \\ <0.5354><0.6669><0.5662><0.5264> \\ <0.6079><0.5971><0.5741><0.5736> \\ <0.5705><0.8376><0.6764><0.5754> \end{bmatrix}$$

(7) Using Definition 4.1, the total value for each cashew nut is calculated and presented as

$$V\left(C_{CO}(P_C^M, Q_C^M)\right) = \begin{bmatrix} 0.5953 \\ 0.5737 \\ 0.5881 \\ 0.6649 \end{bmatrix}$$

(8) Arranging the total score, we obtain the ranking of the cashew nuts as below

Cashews	Total score	Rank
c_1	0.5821	II
c_2	0.5737	IV
c_3	0.5881	III
c_4	0.6649	I

The above results shows that, c_4 cashew nuts ranks first and it will be the best cashew nuts in the market.

CONCLUSION

In this paper, we propose correlation coefficient for CPFSMs. We have also discussed some theoretical approaches on the correlation coefficient of CPFSMs. In final, we discussed an algorithm and a case study for the selection of the best cashews available in the market.

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RESEARCH ARTICLE

Green Hydrogen: Future Fuel?

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ABSTRACT

"Green Hydrogen is the solution to India's fuel Problem".
--NitinGadkari (Minister of Road Transport and Highways of India)

"Green hydrogen could be a critical enabler of the global transition to sustainable energy and net zero emissions economies". ---
Dr Emanuele Taibi, Head of the Power Sector Transformation Strategies, International Renewable Energy Agency (IRENA)

Keywords: Green hydrogen, solution, energy, global transition

INTRODUCTION

India has installed 100GW renewable energy capacity, inching closer to target, government announces . Prime Minister Narendra Modi promises 500GW renewable energy to meet India's 50% energy requirement by 2030[2].In March 2020, Nitin Gadkari reaches parliament in hydrogen powered car. The car, a Toyota Mirai, is first of its kind in the country and is part of a pilot project to study its effectiveness on Indian roads and climatic conditions . Rather than gimmick, it was clear indication to people of India that hydrogen fuel is the future as an alternate energy space and can create 50 to 60 lakhs of jobs. It not just government, Mr. Mukesh Ambani's $ 75 billion plan aims to make India a hydrogen hub while Adani group target to invest $ 70 billion in renewable energy and to produce cheapest hydrogen in world. Tata group already looking and developing hydrogen based buses and to deliver 15 hydrogen powered busses within year. So the question is what is this green hydrogen all about? How it is going to play out if we already have lithium based battery as source of energy fuel? Most important as an investor what are the challenges and factors that need to consider before invest in green hydrogen related stock (Veziroglu & Sahin , 2008)?

Challenge for India

There are three specific factors which is matter of concern

1) We are extremely dependent on the Middle East for Oil and thus make us vulnerable to oil shocks and global turmoil.

2) World leaders realize that the climate changes are need to control so India along with 193 other nations signed something called Paris Pact, where India and such members have to cut down greenhouse emission by 50% by 2030.

3) Three of the most important reason is that India does not yet have viable energy substitutes to industries like steel, transmission and chemical.

This means that the existing renewable energy production cannot run these industries profitably(M, 2008). So whoever manages to crack the code of renewable energy will become the net exporter of renewable energy. Thus, eventually they would go to command extraordinary power in the global politics and one of the most important sources is green hydrogen, this is because it can produce very huge amount of energy with zero emission.

Green Hydrogen: Source of Potential

Hydrogen production happens in many ways, based on methods and source of production, hydrogen is categorized into three major categories: Grey hydrogen, blue hydrogen and green hydrogen. Green hydrogen is produced by electrolysis of water which produces oxygen and hydrogen gas as product. The hydrogen produced is used as carrier of energy through which energy is extracted. In the figure-1, it can be seen that from 2009 to 2019, the cost of production of electricity using coal as source of energy has decreased by just 2% from $ 111 to $ 109 per megawatt output power. The cost of nuclear has increased by 26% from $ 123 to $ 155, whereas, the cost of onshore wind power has dropped by 70% going from $ 135 to just $ 41 per megawatt and the cost of solar energy has decreased by 89% going from $ 359 to $ 40 per megawatt energy. So first time in human history, renewable energy source looks cheaper than the fossil fuels and in next ten years this cost is expected to drop further from $ 40 to $ 5. This is the reason why suddenly electrolysis method using hydrogen fuel looks the best solution for cheapest possible source of energy for most important steel, transport and chemical industry.

For Transport

Range and Volume Occupancy: the latest EV Vehicles with advanced lithium based battery, can achieve 400 to 500 km range but these battery will take up 400 to 600 litres of space in car and thus will increase the size of car, while the hydrogen based vehicles will take up less than 50% of the space and in next 5 to 10 years, considering the advancement in hydrogen technology, hydrogen fuel will take only 100 litres of space and can take a range of 480 km to 500 km i.e. 4 times less occupancy space with same range of lithium battery.

Charging Time

EVs like Tesla model battery give 400km range with half an hour charging while other regular battery require 4 to 8 hours of charging. Whereas the hydrogen based vehicles get recharged within less than 10 minutes with same range of distance. This is going to be massive game changer for commercial vehicles like trucks, buses etc(Behling, Williams, & Managi, 2015).

For Steel Industry

This is where India needs to be extremely considerable for something called “Carbon Border Tax” ie carbon must have its price, because nature cannot pay this price anymore(Dougherty, et al., 2008). This means that if countries do not go as far as US or EU to reduce carbon emission or refuse to go in the right direction to reduce carbon emission, then companies of those countries cannot make business with western countries(Advanced Fuel Cells Technology Collaboration, 2020). The countries like India, China and other Asian countries have less stringent environmental rules, makes to produce products at cheaper, in that case EU and US will charge additional tax on products and services if these countries have carbon emission beyond certain level. This will leads to increase in cost of trades and with just USD 30 per metric ton CO2emission could bring down the profitability of steel industry in India by 20%. As India is 2nd largest producer of steel, mostly to European Union and this is going to hurt at large.

For Chemical Industry

Hydrogen is an important raw material in many chemical industries. Blue and Grey hydrogen is supposed to be replaced by green hydrogen and is going to take place with evolution of solar and wind energy(Caglayan, 2019). Similarly hydrogen gas, which is byproduct of energy, has immense application in oil refinery, glass industry, and semi-conductors.

Challenges

Electricity can be supplied from a renewable energy plant directly connected to the electrolyser, from the grid, or from a mix of the two(Kelly, Gibson, & Ouwerkerk, 2008). Using only electricity from a renewable energy plant ensures that the hydrogen is "green" in any given moment. Grid-connected electrolysers can produce for more hours, reducing the cost of hydrogen. However, grid electricity may include electricity produced from fossil fuel plants, so any CO2 emissions associated with that electricity will have to be considered when evaluating the sustainability of hydrogen(Reiter & Lindorfer, 477–489). If we are not using renewable- generated electricity to power the electrolysis process to generate green hydrogen, then we are still having a high environmental impact . Cost of production of hydrogen energy is considerably high, and we expect that with advancement in technology, the cost of production will reduce (Kim, Lee, & Moon, 2008). Currently, hydrogen storage requires extremely high pressure and is therefore too expensive and inefficient for widespread use in vehicles. Green hydrogen incurs significant energy losses at each stage of the value chain. About 30-35% of the energy used to produce hydrogen through electrolysis is lost (IRENA, 2019).

Moving Ahead

Australia is developing 5 gigawatt hydrogen project to harness green energy, mega electrolyser will be powered by combination of solar and wind energy and use desalinated water taken from ocean. UK is planning to use its offshore wind power for a 5GW hydrogen production capacity. Air Products, ACWA Power and NEOM sign agreement for USD 5 billion production facilities in NEOM powered by renewable energy to export green hydrogen energy. Moving ahead, many works at grass root is to be explored mainly in policy making with national and international strategy formulation. Improvement is required in governance systems to develop infrastructure and enabling policies.

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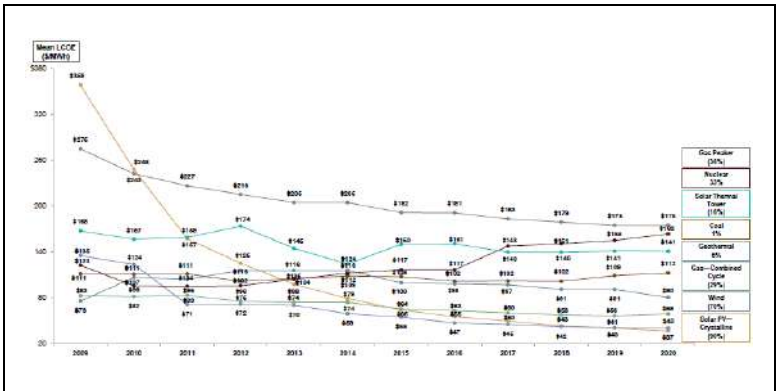

Fig. 1: cost of generation of Electricity [5]

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RESEARCH ARTICLE

A New Area Biased Ram Awadh Distribution with Applications in Medical Science

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ABSTRACT

In this paper, a new generalization of Ram Awadh distribution is proposed known as area biased Ram Awadh distribution. Its different statistical properties including its moments, survival function, hazard rate function, reverse hazard rate function, order statistics, entropies, bonferroni and Lorenz curves have been discussed. Its parameters have also been estimated by using the technique of maximum likelihood estimation. Finally, a real-life data set has been used to illustrate the supremacy of a newly proposed model.

Keywords: Ram Awadh distribution, Weighted distribution, Order statistics, Survival analysis, Maximum likelihood estimation.

INTRODUCTION

The theory of weighted distributions was proposed firstly by Fisher (1934) to model the ascertainment bias. Later it was introduced and formulated in a unified terms by Rao (1965) while modeling statistical data, when the usual practice of using standard distributions was found to be inappropriate. The weighted distributions were formulated in such a situation to record the observation according to some weight function. The weighted distributions provide an adequate approach to deal with model specification and data interpretation. The weighted distributions are used as a tool in selection of appropriate models for observed data, especially when samples are drawn without a proper frame. The weighted distributions provide a technique for fitting models to the unknown weight function when the samples can be taken both from original distribution and developed distribution. The weighted distributions are applied in various research areas related to reliability, biomedicine, ecology, analysis of family data, meta analysis, analysis of intervention data and other areas for the improvement of proper statistical models. The weighted

distributions play a major role in analysing and modeling lifetime data in many applied sciences like engineering, medicine, behavioural sciences, finance and insurance. The weighted distributions are utilised to modulate the probabilities of events as observed and transcribed. The weighted distribution reduces to length biased distribution when the weight function considers only the length of units of interest. The concept of length biased sampling was introduced by Cox (1969) and Zelen (1974). More generally, when the sampling mechanism selects units with probability proportional to measure of the unit size, resulting distribution is called size biased. Size biased distributions are a special case of weighted distributions. A lot of work has been done by many researchers to develop some important weighted probability models with their significant role in handling data sets from various practical fields. Moniem and Diab (2018) discussed on the length-biased weighted exponentiated Lomax distribution. Vijayakumar et al. (2020) presented the length biased Rani distribution with survival data analysis. Elfattah et al. (2021) discussed on the length biased Burr-XII distribution with properties and applications. Shanker et al. (2019) discussed on weighted quasi Lindley distribution with properties and applications.

Perveen et al. (2016) presented area biased weighted Weibull distribution. Subramanian and Shenbagaraja (2019) discussed length biased Rama distribution with bladder cancer data. Mustafa and Khan (2022) studied the length biased powered inverse Rayleigh distribution with applications. Elangovan and Mohanasundari (2019) proposed area biased Aradhana distribution. Shafi et al. (2021) obtained three parameter weighted Pranav distribution with application of relief times, waiting times and carbon fiber. Ahmed et al. (2013) studied size-biased generalized beta distribution of first kind. Fazal (2018) studied area biased poisson exponential distribution with applications. Sharma et al. (2018) discussed on length and area-biased Maxwell distributions. Bashir and Rasul (2016) obtained poisson area-biased Lindley distribution with application on biological data. Recently Ade et al. (2020) presented area biased generalized uniform distribution with some statistical properties. Ram Awadh distribution introduced by Shukla (2018) is a newly proposed one parameter lifetime distribution. Its various statistical properties including its moments, hazard rate function, mean residual function, coefficient of skewness, kurtosis, coefficient of variation, index of dispersion, stochastic ordering, mean deviations, bonferroni and lorenz curves, order statistics, Renyi entropy and stress-strength reliability have been discussed. Its parameters have also been estimated by using the method of moments and method of maximum likelihood estimation. Lastly the goodness of fit test of Ram Awadh distribution has been illustrated with real lifetime data set and the fit was found quite satisfactory over exponential, Lindley, Sujatha, Ishita, Akash, Shanker and Pranav distributions.

Area Biased Ram Awadh (ABRA) Distribution

The probability density function of Ram Awadh (RA) distribution is given by

$$f(x;\lambda)=\frac{\lambda^6}{\lambda^6+120}\left(\lambda+x^5\right)e^{-\lambda x};\ \ x>0,\lambda>0 \qquad (1)$$

and the cumulative distribution function of Ram Awadh distribution is given by

$$F(x;\lambda)=1-\left(1+\frac{\lambda x\left(\lambda^4x^4+5\lambda^3x^3+20\lambda^2x^2+60\lambda x+120\right)}{\lambda^6+120}\right)e^{-\lambda x};\ x>0,\lambda>0 \qquad (2)$$

Let *X* be a random variable following non-negative condition with probability density function $f(x)$ .Let $w(x)$ be the weight function which is a non-negative function, then the probability density function of the weighted random variable X_w is given by

$$f_w(x)=\frac{w(x)f(x)}{E(w(x))},\ \ x>0.$$

Where *w(x)* be a non-negative weight function and $E(w(x))=\int w(x)f(x)dx<\infty.$

Depending upon the choice of the weight function *w(x),* we have different models. Clearly when *w(x) = x*, the resulting distribution is called length biased or size biased. In this paper, we have to obtain the area biased version of

Ram Awadh distribution, so consequently the weight function at $w(x) = x^2$ to obtain the area biased Ram Awadh model. The probability density function of area biased distribution is given by

$$f_a(x) = \frac{x^2 f(x)}{E(x^2)} \tag{3}$$

Where $E(x^2) = \int_0^\infty x^2 f(x)dx$

$$E(x^2) = \frac{2\lambda^6 + 5040}{\lambda^2(\lambda^6 + 120)} \tag{4}$$

Using equations (1) and (4) in equation (3), we will get the probability density function of area biased Ram Awadh distribution as

$$f_a(x) = \frac{\lambda^8}{2\lambda^6 + 5040} x^2\left(\lambda + x^5\right)e^{-\lambda x} \tag{5}$$

and the cumulative distribution function of area biased Ram Awadh distribution can be obtained as

$$F_a(x) = \int_0^x f_a(x)dx$$

$$F_a(x) = \int_0^x \frac{\lambda^8}{2\lambda^6 + 5040} x^2\left(\lambda + x^5\right)e^{-\lambda x}dx$$

$$F_a(x) = \frac{1}{2\lambda^6 + 5040}\int_0^x x^2\lambda^8\left(\lambda + x^5\right)e^{-\lambda x}dx$$

After simplification of above equation, we will obtain the cumulative distribution function of area biased Ram Awadh distribution as

$$F_a(x) = \frac{1}{2\lambda^6 + 5040}\left(\lambda^6\gamma(3,\ \lambda x) + \gamma(8,\ \lambda x)\right) \tag{6}$$

Survival Analysis

In this section we will discuss the survival function, hazard rate and reverse hazard rate functions of the area biased Ram Awadh distribution.

Survival function

The survival function is also known as reliability function is defined as the probability that a system survives beyond a specified time. The survival function of proposed model can be obtained as

$$S(x) = 1 - F_a(x)$$

$$S(x) = 1 - \frac{1}{2\lambda^6 + 5040}\left(\lambda^6\gamma(3,\ \lambda x) + \gamma(8,\ \lambda x)\right)$$

Hazard function

The hazard function is also known as hazard rate or instantaneous failure rate or force of mortality and is given by

$$h(x) = \frac{f_a(x)}{1 - F_a(x)}$$

$$h(x)=\frac{\lambda^8\,x^2\left(\lambda+x^5\right)e^{-\lambda x}}{(2\lambda^6+5040)-(\lambda^6\gamma(3,\,\lambda x)+\gamma(8,\,\lambda x))}$$

Reverse hazard function

The reverse hazard function is given by

$$h_r(x)=\frac{f_a(x)}{F_a(x)}$$

$$h_r(x)=\frac{\lambda^2\,x^2\left(\lambda+x^5\right)e^{-\lambda x}}{\gamma(3,\,\lambda x)+\gamma(8,\,\lambda x)}$$

Statistical Measures

In this section, we will discuss various statistical properties of area biased Ram Awadh distribution especially its moments, harmonic mean, MGF and characteristic function.

Moments

Let X be the random variable represents area biased Ram Awadh distribution with parameter λ then, the r^{th} order moment $E(X^r)$ of area biased Ram Awadh distribution can be obtained as

$$E\left(X^r\right)=\mu_r{}'=\int_0^\infty x^r\,f_a(x)dx$$

$$E(X^r)=\int_0^\infty x^r\,\frac{\lambda^8}{2\lambda^6+5040}\,x^2\left(\lambda+x^5\right)e^{-\lambda x}dx$$

$$E(X^r)=\frac{\lambda^8}{2\lambda^6+5040}\int_0^\infty x^{r+2}\left(\lambda+x^5\right)e^{-\lambda x}dx$$

$$E(X^r)=\frac{\lambda^8}{2\lambda^6+5040}\left(\lambda\int_0^\infty x^{(r+3)-1}e^{-\lambda x}dx+\int_0^\infty x^{(r+8)-1}e^{-\lambda x}dx\right)$$

After simplification of above equation, we get

$$E(X^r)=\mu_r{}'=\frac{\lambda^6\Gamma(r+3)+\Gamma(r+8)}{\lambda^r(2\lambda^6+5040)} \qquad (7)$$

Substituting r = 1,2, 3 and 4 in equation (7), we will get the first four moments of area biased Ram Awadh distribution as

$$E(X)=\mu_1{}'=\frac{6\lambda^6+40320}{\lambda(2\lambda^6+5040)}$$

$$E(X^2)=\mu_2{}'=\frac{24\lambda^6+362880}{\lambda^2(2\lambda^6+5040)}$$

$$E(X^3)=\mu_3{}'=\frac{120\lambda^6+3628800}{\lambda^3(2\lambda^6+5040)}$$

$$E(X^4) = \mu_4' = \frac{720\lambda^6 + 39916800}{\lambda^4(2\lambda^6 + 5040)}$$

$$\text{Variance} = \frac{24\lambda^6 + 362880}{\lambda^2(2\lambda^6 + 5040)} - \left(\frac{6\lambda^6 + 40320}{\lambda(2\lambda^6 + 5040)}\right)^2$$

$$S.D(\sigma) = \sqrt{\left(\frac{24\lambda^6 + 362880}{\lambda^2(2\lambda^6 + 5040)} - \frac{(6\lambda^6 + 40320)^2}{\lambda^2(2\lambda^6 + 5040)^2}\right)}$$

Harmonic mean

The harmonic mean of the proposed area biased Ram Awadh distribution can be obtained as

$$H.M = E\left(\frac{1}{x}\right) = \int_0^\infty \frac{1}{x} f_a(x)dx$$

$$H.M = \int_0^\infty \frac{\lambda^8}{2\lambda^6 + 5040} x\left(\lambda + x^5\right)e^{-\lambda x}dx$$

$$H.M = \frac{\lambda^8}{2\lambda^6 + 5040}\left(\lambda\int_0^\infty x e^{-\lambda x}dx + \int_0^\infty x^6 e^{-\lambda x}dx\right)$$

$$H.M = \frac{\lambda^8}{2\lambda^6 + 5040}\left(\lambda\int_0^\infty x^{3-2} e^{-\lambda x}dx + \int_0^\infty x^{7-1} e^{-\lambda x}dx\right)$$

After simplification, we get

$$H.M = \frac{\lambda^8}{2\lambda^6 + 5040}\left(\lambda\gamma(3,\lambda x) + \gamma(7,\lambda x)\right)$$

Moment Generating Function and Characteristics Function

Let *X* denotes the random variable following area biased Ram Awadh distribution with parameter *λ*, then the moment generating function of area biased Ram Awadh distribution can be obtained as

$$M_X(t) = E\left(e^{tx}\right) = \int_0^\infty e^{tx} f_a(x)dx$$

$$M_x(t) = \int_0^\infty \left(1 + tx + \frac{(tx)^2}{2!} + ...\right) f_a(x)dx$$

$$M_x(t) = \int_0^\infty \sum_{j=0}^\infty \frac{t^j}{j!} x^j f_a(x)dx$$

$$M_x(t) = \sum_{j=0}^\infty \frac{t^j}{j!}\mu_j'$$

$$M_x(t) = \sum_{j=0}^\infty \frac{t^j}{j!}\left(\frac{\lambda^6\Gamma(j+3) + \Gamma(j+8)}{\lambda^j(2\lambda^6 + 5040)}\right)$$

$$M_X(t) = \frac{1}{(2\lambda^6 + 5040)} \sum_{j=0}^{\infty} \frac{t^j}{j!\lambda^j} \left(\lambda^6 \Gamma(j+3) + \Gamma(j+8)\right)$$

Similarly, the characteristic function of proposed area biased Ram Awadh model can be obtained as

$$\varphi_x(t) = M_X(it)$$

$$M_X(it) = \frac{1}{(2\lambda^6 + 5040)} \sum_{j=0}^{\infty} \frac{it^j}{j!\lambda^j} \left(\lambda^6 \Gamma(j+3) + \Gamma(j+8)\right)$$

Order Statistics

Order statistics make their appearance in many statistical theory and practice. Let's we know that if $X_{(1)}$, $X_{(2)}$,…, $X_{(n)}$denotes the order statistics of a random sample X_1, X_2,…, X_n drawn from a continuous population with probability density function $f_x(x)$and cumulative distribution function $F_x(x)$,then the probability density function of r^{th} order statistics $X_{(r)}$is given by

$$f_{X(r)}(x) = \frac{n!}{(r-1)!(n-r)!} f_X(x)\left(F_X(x)\right)^{r-1}\left(1-F_X(x)\right)^{n-r} \qquad (8)$$

Using equations (5) and (6) in equation (8), we will get the probability density function of r^{th} order statistics of area biased Ram Awadh distribution as

$$f_{X(r)}(x) = \frac{n!}{(r-1)!(n-r)!} \left(\frac{\lambda^8}{2\lambda^6 + 5040} x^2 \left(\lambda + x^5\right) e^{-\lambda x} \right)$$

$$\times \left(\frac{1}{2\lambda^6 + 5040} \left(\lambda^6 \gamma(3, \lambda x) + \gamma(8, \lambda x)\right) \right)^{r-1}$$

$$\times \left(1 - \frac{1}{2\lambda^6 + 5040} \left(\lambda^6 \gamma(3, \lambda x) + \gamma(8, \lambda x)\right) \right)^{n-r}$$

Therefore, theprobability density function ofhigherorderstatistic $X_{(n)}$ of area biased Ram Awadh distribution can be obtained as

$$f_{X(n)}(x) = \left(\frac{n\lambda^8}{2\lambda^6 + 5040} x^2 \left(\lambda + x^5\right) e^{-\lambda x} \right)$$

$$\times \left(\frac{1}{2\lambda^6 + 5040} \left(\lambda^6 \gamma(3, \lambda x) + \gamma(8, \lambda x)\right) \right)^{n-1}$$

and the probability density function of I^{st} order statistic$X_{(1)}$ of area biased Ram Awadh distribution can be obtained as

$$f_{X(1)}(x) = \left(\frac{n\lambda^8}{2\lambda^6 + 5040} x^2 \left(\lambda + x^5\right) e^{-\lambda x} \right)$$

$$\times \left(1 - \frac{1}{2\lambda^6 + 5040} \left(\lambda^6 \gamma(3,\ \lambda x) + \gamma(8,\ \lambda x) \right) \right)^{n-1}$$

Likelihood Ratio Test

Suppose the random sample $X_1, X_2, \ldots, X_n$ of size n drawn from the area biased Ram Awadh distribution. we set-up the hypothesis for testing

$$H_o : f(x) = f(x;\lambda) \qquad \text{against} \qquad H_1 : f(x) = f_a(x;\lambda)$$

In order to investigate, whether the random sample of size n comes from the Ram Awadh distribution or area biased Ram Awadh distribution, the following test statistic is used:

$$\Delta = \frac{L_1}{L_0} = \prod_{i=1}^{n} \frac{f_a(x;\lambda)}{f(x;\lambda)}$$

$$\Delta = \frac{L_1}{L_0} = \prod_{i=1}^{n} \left(\frac{\lambda^2 {x_i}^2 (\lambda^6 + 120)}{2\lambda^6 + 5040} \right)$$

$$\Delta = \frac{L_1}{L_0} = \left(\frac{\lambda^2 (\lambda^6 + 120)}{2\lambda^6 + 5040} \right)^n \prod_{i=1}^{n} {x_i}^2$$

We should reject the null hypothesis, if

$$\Delta = \left(\frac{\lambda^2 (\lambda^6 + 120)}{2\lambda^6 + 5040} \right)^n \prod_{i=1}^{n} {x_i}^2 > k$$

Obviously, we also reject the null hypothesis where

$$\Delta^* = \prod_{i=1}^{n} {x_i}^2 > k \left(\frac{2\lambda^6 + 5040}{\lambda^2 (\lambda^6 + 120)} \right)^n$$

$$\Delta^* = \prod_{i=1}^{n} {x_i}^2 > k^*, \text{Where } k^* = k \left(\frac{2\lambda^6 + 5040}{\lambda^2 (\lambda^6 + 120)} \right)^n$$

When a sample is large of size$n$, $2log\ \Delta$ is distributed as chi-square distribution with one degree of freedom and also p-value is obtained from the chi-square distribution. Thus, we reject the null hypothesis, when the probability value is given by

$p\left(\Delta^* > \theta^*\right)$, Where $\theta^* = \prod_{i=1}^{n} {x_i}^2$ is less than a specified level of significan ce and $\prod_{i=1}^{n} {x_i}^2$ is the observed value of the statistic Δ^*.

Bonferroni and Lorenz Curves

The bonferroni and Lorenz curves are used in economics to study the distribution of inequality in income or poverty, but nowadays it is also being used in other fields like reliability, medicine, insurance and demography. The bonferroni and Lorenz curves are given by

$$B(p)=\frac{1}{p\mu_1'}\int_0^q xf(x)dx$$

and $L(p)=\frac{1}{\mu_1'}\int_0^q xf(x)dx$

Where $\mu_1'=\frac{6\lambda^6+40320}{\lambda(2\lambda^6+5040)}$ and $q=F^{-1}(p)$

$$B(p)=\frac{\lambda(2\lambda^6+5040)}{p(6\lambda^6+40320)}\int_0^q \frac{\lambda^8}{2\lambda^6+5040}x^3\left(\lambda+x^5\right)e^{-\lambda x}dx$$

After simplification, we get

$$B(p)=\frac{\lambda^9}{p(6\lambda^6+40320)}\left(\lambda\gamma(4,\ \lambda q)+\gamma(9,\ \lambda q)\right)$$

$$L(p)=\frac{\lambda^9}{(6\lambda^6+40320)}\left(\lambda\gamma(4,\ \lambda q)+\gamma(9,\ \lambda q)\right)$$

Entropy

The term entropy is important in different fields such as probability and statistics, physics, communication theory and economics. Entropies quantify the diversity, uncertainty, or randomness of a system. Entropy of a random variable *X* is a measure of variation of uncertainty.

Renyi Entropy

The Renyi entropy is important in ecology and statistics as index of diversity. The Renyi entropy is also important in quantum information, where it can be used as a measure of entanglement. For a given probability distribution, Renyi entropy is given by

$$e(\alpha)=\frac{1}{1-\alpha}\log\left(\int {f_a}^{\alpha}(x)\,dx\right)$$

Where, $\alpha>0$ and $\alpha\neq1$

$$e(\alpha)=\frac{1}{1-\alpha}\log\int_0^\infty\left(\frac{\lambda^8}{2\lambda^6+5040}x^2\left(\lambda+x^5\right)e^{-\lambda x}\right)^{\alpha}dx$$

$$e(\alpha)=\frac{1}{1-\alpha}\log\left(\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\alpha}\int_0^\infty x^{2\alpha}e^{-\lambda\alpha\, x}\left(\lambda+x^5\right)^{\alpha}dx\right) \qquad (9)$$

Using binomial expansion in equation (9), we get

$$e(\alpha)=\frac{1}{1-\alpha}\log\left(\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k}\lambda^{\alpha-k}x^{5k}\int_0^\infty x^{2\alpha}e^{-\lambda\alpha\, x}dx\right)$$

$$e(\alpha)=\frac{1}{1-\alpha}\log\left(\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k}\lambda^{\alpha-k}\int_0^\infty x^{(2\alpha+5k+1)-1}e^{-\lambda\alpha\, x}dx\right)$$

$$e(\alpha)=\frac{1}{1-\alpha}\log\left(\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\alpha}\sum_{k=0}^{\infty}\binom{\alpha}{k}\lambda^{\alpha-k}\frac{\Gamma(2\alpha+5k+1)}{(\lambda\alpha)^{2\alpha+5k+1}}\right)$$

Tsallis Entropy

The generalization of Boltzmann-Gibbs (B.G) statistical mechanics initiated by Tsallis has focused a great deal to attention. This generalization of B-G statistics was proposed firstly by introducing the mathematical expression of Tsallis entropy (Tsallis, 1988) for a continuous random variable is defined as follows

$$S_\theta=\frac{1}{\theta-1}\left(1-\int_0^{\infty}{f_a}^{\theta}(x)dx\right)$$

$$S_\theta=\frac{1}{\theta-1}\left(1-\int_0^{\infty}\left(\frac{\lambda^8}{2\lambda^6+5040}x^2\left(\lambda+x^5\right)e^{-\lambda x}\right)^{\theta}dx\right)$$

$$S_\theta=\frac{1}{\theta-1}\left(1-\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\theta}\int_0^{\infty}x^{2\theta}e^{-\lambda\theta x}\left(\lambda+x^5\right)^{\theta}dx\right) \qquad (10)$$

Using binomial expansion in equation (10), we get

$$S_\theta=\frac{1}{\theta-1}\left(1-\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\theta}\sum_{k=0}^{\infty}\binom{\theta}{k}\lambda^{\theta-k}x^{5k}\int_0^{\infty}x^{2\theta}e^{-\lambda\theta x}dx\right)$$

$$S_\theta=\frac{1}{\theta-1}\left(1-\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\theta}\sum_{k=0}^{\infty}\binom{\theta}{k}\lambda^{\theta-k}\int_0^{\infty}x^{(2\theta+5k+1)-1}e^{-\lambda\theta x}dx\right)$$

$$S_\theta=\frac{1}{\theta-1}\left(1-\left(\frac{\lambda^8}{2\lambda^6+5040}\right)^{\theta}\sum_{k=0}^{\infty}\binom{\theta}{k}\lambda^{\theta-k}\frac{\Gamma(2\theta+5k+1)}{(\lambda\alpha)^{2\theta+5k+1}}\right)$$

Maximum Likelihood Estimation and Fisher's Information Matrix

In this section, we will discuss the parameter estimation of area biased Ram Awadh distribution by using the technique of maximum likelihood estimation and also obtain its Fisher's information matrix. Consider $X_1, X_2,\ldots, X_n$ be the random sample of size n from the area biased Ram Awadh distribution, then the likelihood function is given by

$$L(x)=\prod_{i=1}^{n}f_a(x)$$

$$L(x)=\prod_{i=1}^{n}\left(\frac{\lambda^8}{2\lambda^6+5040}{x_i}^2\left(\lambda+{x_i}^5\right)e^{-\lambda x_i}\right)$$

$$L(x)=\frac{\lambda^{8n}}{(2\lambda^6+5040)^n}\prod_{i=1}^{n}\left({x_i}^2\left(\lambda+{x_i}^5\right)e^{-\lambda x_i}\right)$$

The log likelihood function is given by

$$\log L=8n\log\lambda-n\log(2\lambda^6+5040)+2\sum_{i=1}^{n}\log x_i+\sum_{i=1}^{n}\log\left(\lambda+{x_i}^5\right)-\lambda\sum_{i=1}^{n}x_i \qquad (11)$$

The maximum likelihood estimate of λ can be obtained by differentiating the log likelihood equation (11) with respect to λ and must satisfy the normal equations as

$$\frac{\partial \log L}{\partial \lambda} = \frac{8n}{\lambda} - n\left(\frac{12\lambda^5}{2\lambda^6 + 5040}\right) + \sum_{i=1}^{n}\left(\frac{1}{(\lambda + x_i^{\,5})}\right) - \sum_{i=1}^{n} x_i = 0$$

The above likelihood equation is too complicated to solve it algebraically. Therefore we use R and wolfram mathematics for estimating the required parameters of the proposed distribution. In order to obtain the confidence interval, we use the asymptotic normality results. we have that if $\hat{\alpha} = \hat{\lambda}$ denotes the MLE of $\alpha = \lambda$ we can state the results as follows:

$\sqrt{n}(\hat{\alpha} - \alpha) \to N(0, I^{-1}(\alpha))$

Where $I(\alpha)$ is Fisher's Information matrix.i.e.,

$$I(\alpha) = -\frac{1}{n}\left(E\left(\frac{\partial^2 \log L}{\partial \lambda^2}\right)\right)$$

Here we define

$$E\left(\frac{\partial^2 \log L}{\partial \lambda^2}\right) = -\frac{8n}{\lambda^2} - n\left(\frac{(2\lambda^6 + 5040)\,60\lambda^4 - 12\lambda^5(12\lambda^5)}{(2\lambda^6 + 5040)^2}\right) - \sum_{i=1}^{n}\left(\frac{1}{(\lambda + x_i^{\,5})^2}\right)$$

Since α being unknown, we estimate $I^{-1}(\alpha)$ by $I^{-1}(\hat{\alpha})$ and this can be used to obtain asymptoticconfidence intervals for λ.

Application

In this section, we have fitted a real lifetime data set in area biased Ram Awadh distribution to discuss its goodness of fit and the fit has been compared over Ram Awadh, exponential and Lindley distributions. The real-life data set is given below in table 1 as: The following real life data set represents 40 patients suffering from blood cancer (leukemia) reported from one of ministry of health hospitals in Saudi Arabia (see Abouammah et al.). The ordered lifetimes (in years) is given below as:In order to estimate the model comparison criterion values, the unknown parameters are also estimated through the R software. In order to compare the area biased Ram Awadh distribution with Ram Awadh, exponential and Lindley distributions, we are using the criterion values *AIC* (Akaike Information Criterion), *BIC* (Bayesian Information Criterion), *AICC* (Akaike Information Criterion Corrected)and *-2 log L*. The better distribution is which corresponds to lesser values of *AIC, BIC, AICC* and *-2logL*. For calculating *AIC, BIC, AICC* and *-2logL* can be evaluating by using the formulas as follows

$$AIC = 2k - 2\log L, \qquad BIC = k\log n - 2\log L \qquad \text{and} \qquad AICC = AIC + \frac{2k(k+1)}{n-k-1}$$

Where *k* is the number of parameters in the statistical model, *n* is the sample size and *-2logL* is the maximized value of log-likelihood function under the considered model. From table 2 given above, it can be clearly observed from the results that the area biased Ram Awadh distribution have the lesser *AIC, BIC, AICC* and *-2logL* values as compared to Ram Awadh, exponential and Lindley distributions. Hence, it can be concluded that the area biased Ram Awadh distribution leads to a better fit as compared to Ram Awadh, exponential and Lindley distributions.

CONCLUSION

In the present study, we have proposed a new generalization of Ram Awadh distribution called as area biased Ram Awadh distribution. The subject distribution is generated by using the area biased technique and taking the Ram Awadh distribution as the base distribution. Its various structural properties including its moments, moment

generating function, characteristic function, hazard rate function, survival function, order statistics, entropies, bonferroni and lorenz curves have been described and discussed. The estimation of parameters have also been discussed by using the method of maximum likelihood estimation and also its Fisher's information matrix have been presented. Finally, the newly proposed distribution has been demonstrated with real life data set to discuss the superiority of area biased Ram Awadh distribution over Ram Awadh, exponential and Lindley distributions.

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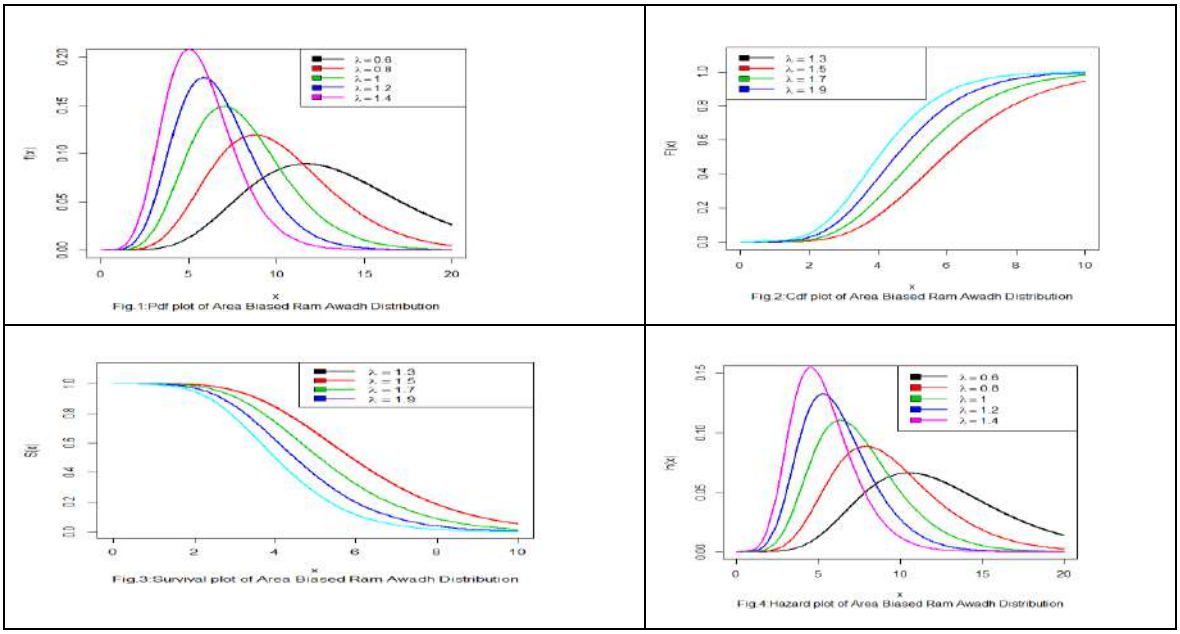
Table 1: Data regarding blood cancer (leukemia) patients reported by Abouammah *et al.* (2000)

0.315	0.496	0.616	1.145	1.208	1.263	1.414	2.025	2.036
2.162	2.211	2.37	2.532	2.693	2.805	2.91	2.912	3.192
3.263	3.348	3.348	3.427	3.499	3.534	3.767	3.751	3.858
3.986	4.049	4.244	4.323	4.381	4.392	4.397	4.647	4.753
4.929	4.973	5.074	5.381					

Table 2: Comparison and Performance of fitted distributions

Distributions	MLE	S.E	-2logL	AIC	BIC	AICC
Area Biased Ram Awadh	$\hat{\lambda} = 2.440271$	$\hat{\lambda} = 0.1208242$	139.942	141.942	143.631	142.047
Ram Awadh	$\hat{\lambda} = 1.6618903$	$\hat{\lambda} = 0.0840995$	141.650	143.650	145.339	143.755
Exponential	$\hat{\lambda} = 0.31839887$	$\hat{\lambda} = 0.0503427$	171.556	173.556	175.245	173.661
Lindley	$\hat{\lambda} = 0.52692132$	$\hat{\lambda} = 0.0607476$	160.5012	162.501	164.19	162.6064



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RESEARCH ARTICLE

Formulation of Microbial Consortium for the Effective Treatment of Sewage Water and its Reuse in Irrigation

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ABSTRACT

The present study was conducted for sewage treatment using an effective microbial consortium. *Lactobacillus*, *Pseudomonas*, *Aspergillus*, and *Saccharomyces* were the effective microorganisms (EM) isolated from respective sources. Under aerobic conditions, sewage treatment was carried out with the addition of various percentages of EM solution. After five days of treatment, BOD, COD, TDS, and TSS were all lowered. The results showed that the formulated EM was effective in treatment of sewage and could reduce the environmental impact of the same. In the phenotypic study, the different concentrations of treated sewage water did not affect the growth of sorghum under controlled condition. Therefore, the treated sewage water could be used for agriculture purpose.

Keywords: Effective Microorganisms; Sewage; BOD; COD; TDS; TSS
Abbreviation: EM: Effective Microorganisms.

INTRODUCTION

Water scarcity in India is becoming a year-round problem that is affecting nearly one million people with a lack of sufficient and safe access to it. Drought conditions and depleting natural water resources are drawing attention to what remains a global issue: a lack of access to safe and drinkable water. With water from various assets being removed for different human exercises, in many parts of the world, water is in short supply. Water is becoming increasingly scarce as it is required to cultivate and prepare food, generate electricity, and support industry for an ever-increasing population. Climate change is a significant contributor. Only 2.5% of the earth's water is represented by freshwater with less than 0.3% of it, a part of rivers, lakes, and the atmosphere (Gleick, 1993). The ecological

implications of water use and pressure are multifaceted; they reduce crop yields and individual well-being, resulting in famine. Several factors contribute to India's water shortage, including a suitable structure, government control, waste water and unchecked contamination.

Sewage water is the waste generating from community's residents. Grey water and black water make up this sewage water with the former coming from bathing, washing dishes, and doing laundry and toilet wastewater refers to as black water. Furthermore, sewage water has a bad odour. Water reuse has been rapidly increasing and some experts consider it to be the greatest challenge of the 21st century (Asano, 2002). As a result, treatment is becoming critical as it guarantees that the water released into local waterways, such as rivers, is safe and clean, with the goal of preventing harm to people and aquatic life. Hence, wastewater reuse has been identified as a promising method for dealing with the global problem of water scarcity (Garcia & Pargament, 2015).

There is no particular strategy for treating the majority of the chemicals occurring in the sewage water in one step. Also, the amount generated is increasing rapidly, and the deteriorating quality of this massive amount of wastewater is outpacing the streams' and rivers' self-purification ability. As a result, different therapeutic methods are being created to accelerate the forces of nature in a controlled environment for the following concerns namely the removal of suspended and floatable material from waste water, the treatment of biodegradable organics (BOD removal), and the elimination of disease-causing pathogenic microorganisms (Rajasulochana & Preethy, 2016). Physical water treatment, which involves filtration and disinfection, is one of four primary approaches to treat wastewater. To remove solids, processes such as screening, sedimentation, and skimming are utilized. Chemical treatment, which involves the use of chemicals in water such as chlorine, sludge treatment, and biological water treatment are also used.

Microbes are vital in terms of the environment, economy, and society. These are currently being used to clean up harmful waste on a large industrial scale. The breakdown of organic compounds in wastewater is primarily carried out by microorganisms and their enzymes and is a less expensive technology. Aside from being a cost-effective and environmentally friendly solution, it also offers the finest alternative to traditional treatment procedures (Rani, Sangwan, Joshi, Sagar, & Bala, 2019).

It is important to define the relations between the microbial species structure and the operational parameters of full-scale wastewater treatment plants (WWTP) as they are the influencing factors behind the formation of complex microbial structures and their species composition and these in turn determine the metabolic pathways in the WWTP and the quality of treated wastewater (A & M, 2016).Effective Microorganism (EM) is a consortium of beneficial, naturally occurring microorganisms that are being integrated in agriculture to stimulate plant development and soil fertility. These microorganisms are neither synthetically blended or hereditarily modified. The EM that were utilized in this investigation includes *Lactobacillus*, *Pseudomonas*, *Aspergillus*, *Saccharomyces* and *Streptomyces*.

Loyola's current total water usage is 6.87 lakhs litres per day. Permanent inmates would require 2,49,750 litres (1850 persons 135 litres) per capita, while day scholars would require 4,05,000 litres (9000 persons 45 litres). With an additional 5% for landscape maintenance and other secondary functions. Since the institution is constantly expanding, future water consumption is expected to rise by 5% every 5 to 10 years, reaching roughly 7.21 lakhs by 2023. The wastewater created by the entire college, which includes sewage, toilet wastes, kitchen wash water, laundry waste, and other garbage, is approximately 1,50,000 litres per day, or 4.5 million litres per month.

The goal of this study was to investigate the physico-chemical standards of wastewater and to test different bacteria against wastewater from the Loyola STP in order to create clean water for crop enhancement. This research would be most beneficial in terms of purifying wastewater and using it to improve agricultural development in communities with water scarcity.

MATERIALS AND METHODS

Collection of samples

Water sample was collected from the Loyola Sewage Treatment Plant, Loyola College, Chennai and brought to the laboratory in a container. Serial dilution was used to isolate bacteria and fungi from the sample, followed by spread plate and streak plate procedures on respective media and incubated at 37ºC for 24 h (Penna, Martins, & Mazzola, 2002)

Culturing of microorganisms

Different media were used to culture the microorganisms. *Lactobacillus* was grown in Methicillin-Resistant *Staphylococcus aureus* (MRSA) enrichment broth, *Saccharomyces* in yeast growth, *Aspergillus* in potato dextrose broth and *Pseudomonas* in Luria broth (Penna et al., 2002)

Physiochemical parameters

Various tests were conducted to determine the Total Dissolved Solids (TDS) and Total Suspended Solids (TSS) (IS: 3025 part 16 & 17), Biochemical oxygen demand (BOD) (IS: 3025 part 44 - reaffirmed 2019) and Chemical oxygen demand (COD) (IS: 3025 part 58 - reaffirmed 2006). All the tests were carried out based on the standard protocol. These tests were performed to ascertain the removal/reduction efficiency of the above-mentioned parameters.

Greenhouse study

Plant material and growth conditions

The sorghum seeds were collected from Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu, India and the study was performed at Entomology Research Institute, Loyola College. The plants were grown in triplicate and growth response was observed under various concentrations of treated sewage water (25%, 50%, 75% and 100%), while maintaining them at the optimal growth conditions (aerated condition at 27 ± 2°C with 16/8 (h) light and dark cycle, and 85% relative humidity). Healthy plants were employed for phenotypic analysis after 15 days.

Plant phenotyping

The phenotypic traits namely shoot length (SL), primary root length (PRL), shoot fresh weight (SFW), shoot dry weight (SDW), root fresh weight (RFW) and root dry weight (RDW) were observed under six different levels of treated sewage water (TSW) (25%,50%,75%,100%) and measured following that of (Ramakrishnan et al., 2017) and (Maharajan, Ceasar, Krishna, &Ignacimuthu, 2019). All data were recorded from one plant selected from each bag, and the mean value was calculated (n=3) using SPSS (Statistical Package for the Social Sciences).

RESULTS AND DISCUSSION

Isolation of bacteria

Around four microorganisms were isolated using the spread plate and streak plate techniques.

Culturing of microorganisms

The microorganisms were cultured using different media.

Physiochemical parameters

Total Dissolved Solids (TDS)

The EM reduced the TDS from 3290 to 3149 mg/l in the control and was effective while treated at concentration of 1% and 3% for 5 days, but the values in the 5%, 7% and 9% indicates that at higher concentration the TDS value increased from day 1 to day 5 (Figure 3). The values obtained where in accordance with the results of Sathiavelu *et al*., 2011.

Total Suspended Solids (TSS)

The TSS of the sewage was reduced from 514.3 to 478.2 mg/l in the control. The EM showed the effective reduction of TSS while treated at concentration of 1% and 3% when treated for 5 days. But the values in the 5%, 7% and 9% indicates that at higher concentration the TSS value increased from day 1 to day 5 (Figure 4). The values obtained where in accordance with the results of (Sirianuntapiboon, Phothilangka, & Ohmomo, 2004).

Biochemical Oxygen Demand (BOD)

An effective result was shown by the EM treated at a concentration of 3 ml/l for 3 days when compared to the control which showed a decrease in BOD from 410.2 to 381.9 mg/l in 3 days whereas in the 1% and 3% at day 3 the value of decrease was much higher from 399.3 to 358.8 mg/l and from 406.3 to 361.4 mg/l respectively (Figure 5). In case of higher concentrations of the sewage water the reduction is not observed, this can be due to the increasing number of bacterial colonies and the subsequent usage of oxygen. Similar results were obtained by (Mongkolthanaruk & Dharmsthiti, 2002) with a bacterial consortium using *Pseudomonas*, *Bacillus* and *Acinetobacter* and molasses for treating lipid rich wastewater resulting in a reduced BOD from 448 to 72 mg/l. (Mohana *et al.,* 2007)used the bacterial consortium of *Pseudomonas aeruginosa, Bacillus megaterium* and *Stenotrophomonas maltophilia* for treating paper and pulp mill effluent and observed BOD reduction from 87 to 89%.

Chemical Oxygen Demand (COD)

The EM reduced the COD effectively while treated at concentration of 3% for 3 days and a decrease in COD was seen from 294.3 to 267.3 mg/l in 5 days with a maximum reduction achieved in 3% and 1% on the day 3. Similar results were obtained by (Samy, Ignacimuthu, Ethnopharmacology, & 1999, n.d.)(Wididana, n.d.)where the EM reduced the COD of wastewater from Nestle and Trebor companies to 76% in 11 days at a concentration 1 ml/l. (Gilliland, 1979) reported a reduced COD by 29% and 37% in 16 and 20 hours on treatment of whey disposed from cheese manufacturing industry using *Kluyveromyces fragilis* (Figure 6).

Greenhouse study and plant phenotyping

The phenotypic traits such as SL, PRL, SFW, SDW, RFW and RDW were observed under four different concentrations of TSW (25%, 50%, 75% and 100%). These could not influence the phenotype of 15-days old seedlings of sorghum plant. The shoot length was 8.83 cm in control (DDH_2O), 7.76 cm in control (GW), 6.83 cm in 25% TSW, 8.23 cm in 50% TSW, 7.26 cm in 75% TSW and 6.76 cm in 100% TSW. The root length was 19.46 cm in control (DDH_2O), 22.33 cm in control (GW), 20.16 cm in 25% TSW, 21.10 cm in 50% TSW, 19.93 cm in 75% TSW and 22.46 cm in 100% TSW. Therefore, the SL and PRL showed no significant variation when compared with the control. This indicated that there were no toxic effects on plant growth by TSW.

The SFW were 552.13 mg, in control (DDH_2O), 635.83 mg in control (GW), 647.30 mg in 25% TSW, 692.73 mg in 50% TSW, 535.96 mg in 75% TSW and 426.36 mg in 100% TSW. The RFW were 97.50 mg in control (DDH_2O), 143.96 mg in control (GW), 122.80 mg in 25% TSW, 195.06 mg in 50% TSW, 116.06 mg in 75% TSW and 222.50 mg in 100% TSW were observed. Therefore, the SFW and RFW showed no huge reduction in plant biomass when compared with the control. The SDW were 84.20 mg, in control (DDH_2O), 101.13 mg in control (GW), 98.70 mg in 25% TSW, 111.90 mg in 50% TSW, 91.06 mg in 75% TSW and 68.90 mg in 100% TSW. The RDW were 20.00 mg, in control (DDH_2O), 29.93 mg in control (GW), 37.76 mg in 25% TSW, 40.13 mg in 50% TSW, 30.33 mg in 75% TSW and 36.93 mg in 100% TSW were observed. Therefore, the SFW, RFW and SDW, RDW showed no huge reduction in plant biomass when compared with the control, although, sufficient growth was seen in 50% and 75%. This indicated that the TSW have no toxic effects on plant growth.

Similar results were obtained by (Khaeim et al., 2020) where optimum growth of plants was observed under 25%-75% without toxicity. (Paliwal, Karunaichamy, & Ananthavalli, 1998)*also studied the effect of sewage water on Hardwickiabinata which were found to be in accordance with the greenhouse studies. Similar results were obtained by (Al-Othman, Ali, Al-Othman, Ali, & A. Habila, 2016) which confirmed the non-toxic effects and reusable attributes of TSW for irrigation purposes.*

CONCLUSION

The Effective Microbial consortium was formulated and its efficiency for sewage treatment was studied. The results showed the reduction of BOD, COD, TDS, and TSS as well as the malodor and turbidity of sewage by the EM treatment. The treatment process was highly viable and economical. The EM-treated water was non-toxic and safe to dispose of as it contains beneficial microorganisms which reduces the environmental impact of conventional methods. Greenhouse studies with TSW showed the absence of toxic effects as all the plants grown there showed no significant variation in their phenotypic responses. However, some plants showed increased root fresh and dry weight. In general, optimum growth conditions were observed in 50% and 75%. This implied that the TSW has no toxic or inhibitory effects on the plants and could be even used for irrigation and agricultural purposes while conserving water. Further studies can be carried out for the formulation of the above consortium as a product to be used on a larger scale in the agricultural field.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1:Mean value on effect of EM treatment on TDS reduction of sewage

	1 Day	2 Day	3 Day	4 Day	5 Day
Control	3290	3178	3163	3154	3149
1%	3170	3134	3005	2940	2900
3%	3014	2900	2874	2843	2700
5%	3250	3160	3294	3310	3260
7%	3265	3250	3230	3242	3295
9%	3210	3255	3284	3319	3342

Table 2: Mean value on effect of EM treatment on TSS reduction of sewage

	1 Day	2 Day	3 Day	4 Day	5 Day
Control	514.3	501.2	492.4	482.6	478.2
1%	523.1	511.3	488.3	481.3	471.3
3%	511.2	492.3	473.4	483.1	485.2
5%	519.7	529.6	524.3	521.3	526.4
7%	514.2	518.7	515.3	521.4	528.6
9%	522.4	529.3	533.9	546.3	559.4

Table 3:Mean value on effect of EM treatment on BOD reduction of sewage

	1 Day	2 Day	3 Day	4 Day	5 Day
Control	410.2	394.2	381.9	377.5	364.3
1%	406.3	383.5	361.4	359.7	369.2
3%	399.3	376.3	358.8	364.3	372.1
5%	411.4	401.3	403.5	402.5	420.9
7%	422.9	423.1	427.4	425.5	429.3
9%	434.3	430.3	436.7	442.3	456.3

Table 4:Mean value on effect of EM treatment on COD reduction of sewage

	1 Day	2 Day	3 Day	4 Day	5 Day
Control	294.3	281.1	267.3	261.8	258.7
1%	299.6	279.2	258.9	257.3	243.1
3%	307.2	274.5	250.3	249.4	259.4
5%	305.1	310.3	309.8	316.5	320.4
7%	319.3	316.2	328.4	326.3	330.1
9%	312.3	320.4	329.6	337.8	331.4

Table5: Growth intervals of sorghum (mean value)grownunder different concertation of TSW

Treated sample	5 Day	10 Day	15 Day
Ctrl (DDH_2O)	6.16 ± 0.4	7 ± 0.43	8.83 ± 0.47
Ctrl (GW)	5.76 ± 0.251	6.43 ± 0.51	7.76 ± 0.25
TSW (25%)	6.1 ± 0.360	6.9 ± 0.1	8.1 ± 0.40
TSW (50%)	6.63 ± 0.757	7.26 ± 0.64	8.23 ± 0.30
TSW (75%)	6.6 ± 0.360	7.16 ± 0.65	8.26 ± 0.40
TSW (100%)	5.6 ± 0.36	6.06 ± 0.11	7.76 ± 0.45

Table 6:Mean value on length of shoot and root of sorghum grown under different concentration of sewage water.

Treated sample	Shoot length	Root length
Ctrl (DDH_2O)	8.83 ± 0.47	19.46 ± 0.75
Ctrl (GW)	7.76 ± 0.25	22.33 ± 1.06
TSW (25%)	6.83 ± 0.4	20.16 ± 0.7
TSW (50%)	8.23 ± 0.3	21.10 ± 0.55
TSW (75%)	7.26 ± 0.41	19.93 ± 0.5
TSW (100%)	6.76 ± 0.45	22.46 ± 1.1

Table 7: Mean value on fresh weight of shoot and root of sorghum grown under different concentration of sewage water.

Treated Sample	SFW	RFW
Ctrl (DDH_2O)	552.13 ± 0.8	97.50 ± 0.6
Ctrl (GW)	635.83 ± 0.2	143.96 ± 0.72
TSW (25%)	647.30 ± 0.55	122.80 ± 0.65
TSW (50%)	692.73 ± 0.85	195.06 ± 0.55
TSW (75%)	535.96 ± 0.95	116.06 ± 0.7
TSW (100%)	426.36 ± 0.66	222.50 ± 0.87

Table 8: Mean value of Dry weight of shoot and root of sorghumgrown under different concentration of sewage water.

Treated sample	SDW	RDW
Ctrl (DDH_2O)	84.20 ± 0.47	20.00 ± 0.75
Ctrl (GW)	101.13 ± 0.25	29.93 ± 1.06
TSW (25%)	98.70 ± 0.4	37.76 ± 0.7
TSW (50%)	111.90 ± 0.3	40.13 ± 0.55
TSW (75%)	91.06 ± 0.41	30.33 ± 0.5
TSW (100%)	68.90 ± 0.45	36.93 ± 1.1

Figure. 1a:Spread plate of *Saccharomyces*

Figure. 1b:Streak plate of *Lactobacillus*

A- *Aspergillus*, B- *Lactobacillus*, C- *Pseudomonas*, D- *Saccharomyces*

Figure. 2:Media for all the four microorganisms

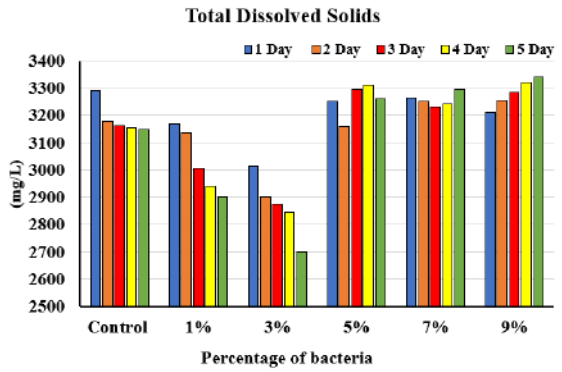

Figure. 3:Effect of EM treatment on TDS reduction of sewage

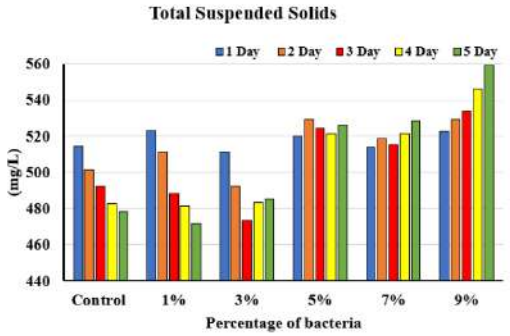

Figure. 4:Effect of EM treatment on TSS reduction of sewage

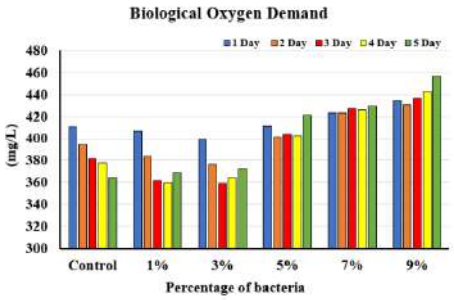

Figure. 5:Effect of EM treatment on BOD reduction of sewage

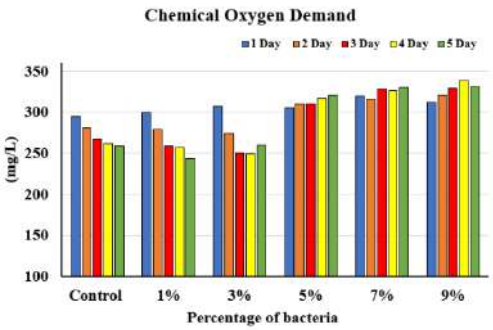

Figure. 6:Effect of EM treatment on COD reduction of sewage

Figure. 7:Plant growth under different concentration of treated sewage water. The figure I, II and III represents 1, 5, 15 days of plant (Sorghum) growth respectively. A- Control (DDH$_2$0); B- Control (GW); C- TSW (25%); D- TSW (50%); E-TSW (75%); F-TSW (100%).

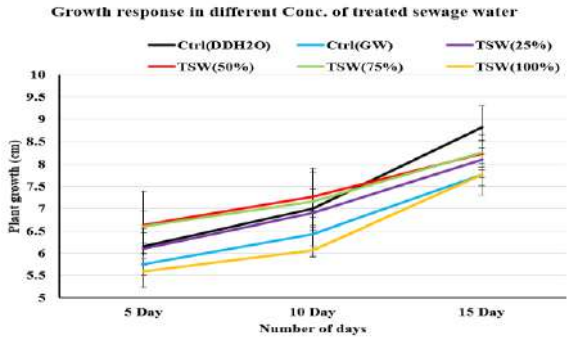

Figure. 8:Growth intervals of sorghumunder different concertation of TSW

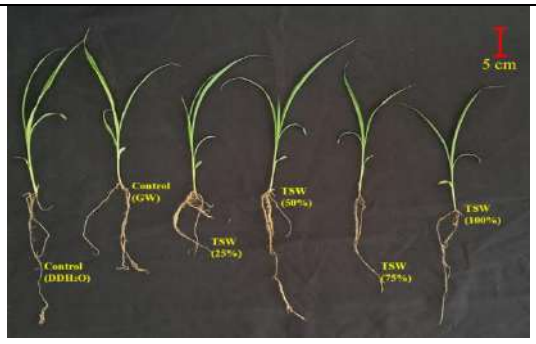

Figure.9:Overall Plant growth response of Sorghumplantgrown under Control (DDH$_2$O), control (GW), TSW (25%), TSW (50%), TSW (75%), and TSW (100%).

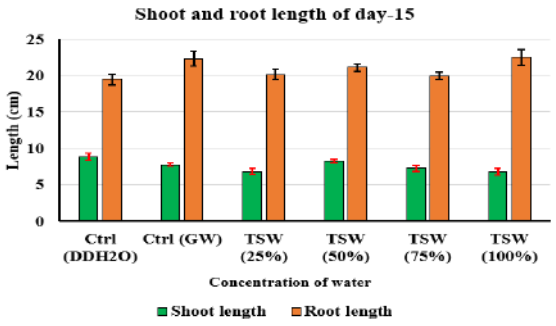

Figure. 10:Plant shoot and root length of day 15under different concentration

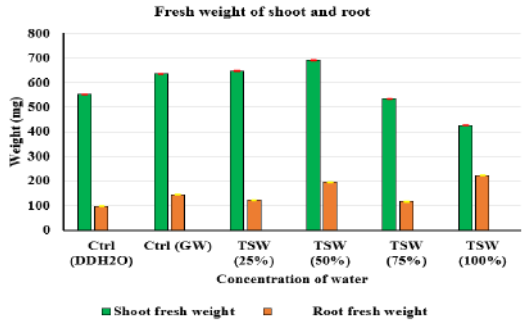

Figure. 11:Fresh weight of shoot and root of sorghum grown under different concentration of sewage water.

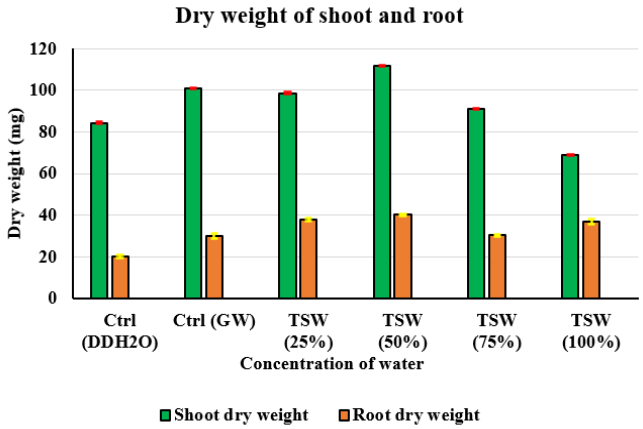

Figure. 12:Dry weight of shoot and root of sorghumgrown under different concentration of sewage water.

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RESEARCH ARTICLE

Inhibition of Corrosion of Carbon Steel in Well Water by Homo Alanine – Zn^{2+} System

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ABSTRACT

In the last two decades, exploration in the field of erosion impediments had been directed towards the thing of using cheap effective motes of low ornon-negative environmental impact to replace the environmentally Homo Alanine composites. One of the encourager composites which can be used as safe erosion impediments are Homo alanine. They're environmentally, friendly, non – poisonous, biodegrable and fairly, cheap. On other hand, the development of computational modeling helps to understand the inhibition medium of those emulsion s and to develop the newely designed impediments. In this review, utmost of donation made in literatue on the use of Homo Alanine and their derivations as erosion impediments for metallic blends accoutrements were presented and bandied. A going together effect has existance Homo Alanine- Zn^2. Homo Alanine – Zn^2 system the rules to make up of 300ppm of Homo Alanine-30 ppm of Zn^2 98. Movement to contrary positions work space gives knowledge of that putting of Ac impedance gamuts gives knowledge of that a defensive filmland rolls is formed on the essence top. The top morphology has been got broken up(into simpler corridor). A right medium of erosion inhibition is offered grounded on the results got from weight loss work space@ electrochemical makes observation about.

Keywords: Carbon steel, Homo Alanine, synergistic effect,

INTRODUCTION

Erosion is further than just an ineluctable natural miracle, its impact is felt in three areas of concern, videlicet profitable, safety, and environmental damage. Metallic erosion, putatively inoffensive, affects numerous sectors. The rudiments of the costs of erosion included capital, design and control as well as associated costs. The high costs of erosion have a significant effect on the public frugality, in general, erosion costs quantum to about 2- 4of gross public

product. Thus, it's necessary that erosion help borrow erosion control measures Utmost essence(with the exception of noble essence similar as Au, Pt, etc.) live in nature in combined forms as their oxides, carbonates, hydroxyl carbonates, sulphides, chlorides and silicates. These are reduced to their metallic countries from their ores, during their birth processes. During birth of essence, considerable quantities of energy are needed. Accordingly, insulated pure essence can be regarded in agitated state(a advanced energy state) than their corresponding ores, and they've a natural tendency to return back to combined state(or lower energy state). Hence, when essence are put into use, in colorful forms, they're exposed essence shells begin to decay (i.e., conversion into further stable essence composites) more or less fleetly, when they come in contact with gassy and/ or liquid terrain(or surroundings). In other words, destruction may be due to direct chemical attack (by the terrain) or electrochemical (i.e., analogous to response in a Daniel cell). Any process of deterioration(or destruction) and consequent loss of a solid, metallic, through an unwanted(or unintentional) chemical or electrochemical attack by its terrain, starting at its face, is called erosion. Therefore, erosion is a process " reverse of birth of essence ". The most familiar illustration of erosion is rusting of iron, when exposed to the atmospheric conditions. During this, a subcaste of sanguine scale and greasepaint of oxide(Fe_3O_4) is formed, and the iron becomes weak. Another common illustration is conformation of green film of introductory carbonate ($CuCO_3$ Cu($OH)^2$) on the face of bobby, when exposed to wettish- air containing carbon dioxide.

Gravity of corrosion problem:

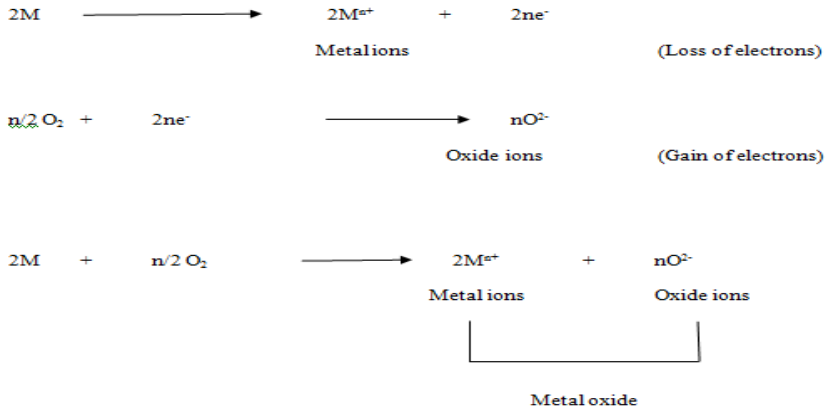
The process of erosion is slow and occurs only at shells of essence, but the losses incurred, due to erosion, are enormous waste/ destruction of machines, accoutrements and different types of metsllic products. Losses being, due to erosion, cannot be measured in terms of the cost of essence alone, but the high cost of fabrication into outfit/ machine tool/ structures should also be considered. The inflexibility of the problem may be made clear by the approximate estimate of loss of essence due to erosion, as 2 to2.5 billion bones per annum each- over the world. Obviously, the mastermind must understand the medium of erosion, if its goods are to be minimized. Also, he'll also be more suitable to avoid oppressively erosion conditions and give contemporaneously protection against erosion.

Dry or Chemical Corrosion

This type of erosion occurs substantially through the direct chemical action of terrain/ atmospheric feasts similar as oxygen, halogen, hydrogen sulphide, sulphur dioxide, nitrogen or anhydrous inorganic liquid with essence shells in immediate propinquity. There are three main type of chemical erosion

Oxidation Corrosion

Oxidation erosion is brought about by the direct action of oxygen at low or high temperatures on essence, in the absence of humidity. At ordinary temperatures, essence,, in general, are veritably slightly attacked. Still, alkali essence(Li, Na, K, Rb,etc.) and alkaline – worlds(Be, Ca, Sr,etc.) are indeed fleetly oxdized at low temperatures. At high temperatures, nearly all essence(except Ag, Au, and Pt) are oxdized. The responses in the oxidation erosion are



Mechanism

Oxidation occurs first at the surface of the metal and the resulting metal oxide scale forms a barrier, that tends to restrict further oxidation. For, oxidation to continue, either the metal must diffuse outwards through the scale to the surface or the oxygen must diffuse inwards through the scale to the underlying metal. Both transfers occur (but the outwards diffusion of metal is, generally, much more rapid than the oxygen ion and consequently, of much higher mobility.

Concentration Cell Corrosion

This type of erosion is due to electromagnetically attack on the essence face, exposed to an electrolyte of varying attention or of varying aeration. This may be the result of original differences or shy agitation or slow prolixity of essence- ions, produced by erosion. Differential aeration erosion (the most common type of attention cell erosion) occurs when one part of essence is exposed to a different air attention from the other part. This causes a difference in eventuality between else aerated areas. It has been set up experimentally that " poor- oxygenated corridor are anodic ". Accordingly, a discrimination aeration of essence causes a inflow of current, called the discrimination current. Differential aeration accounts for the erosion of essence, incompletely immersed in a result, just below the waterline. therefore, if a essence(say Zn) is incompletely immersed in a dilute result of a neutral swab(say NaCl) and the result isn't agitated duly, also, the corridor over and nearly conterminous to the waterline are more explosively aerated(because of the easy access of oxygen) and hence, come cathodic. On the other hand, corridor immersed to lesser depth(which have lower access of oxygen) show a lower oxygen attention and therefore, come anodic. So, a difference of eventuality is created, which causes a inflow of current between the two differential- aerated areas of the same essence. Zinc will dissolves at the anodic areas, and oxygen will take up electrons at the cathodic areas to form hydroxyl ions.

$$Zn \longrightarrow Zn^{2+} + 2e^- \qquad \text{(Oxidation)}$$

$$\tfrac{1}{2}O_2 + H_2O + 2e^- \longrightarrow 2OH^- \qquad \text{(Reduction)}$$

The circuit is completed by migration of ions, through the electrolyte, and flow of electrons, through the metal, from anode to cathode. In a similar way, iron cathodes under drops of water (or salt solution). Areas covered by droplets, having no access of oxygen, becomes anodic with respect to the other areas, which are freely exposed to air. From the above, it is clear that oxygen concentration cell increases corrosion, but it occurs where the oxygen concentration is lower.

General Facts about Differential Aeration Corrosion:

(i) erosion may be accelerated in supposedly inapproachable places, because the oxygen-deficient areas serve as anodes and, thus, cracks or crannies serve as foci for erosion.(ii) erosion is accelerated under accumulation of dirt beach, scale or other attention. This is because accumulation of rust or scale or beach, etc. restricts the access of oxygen and establishes an anode to promote still lesser accumulation. The result is localized erosion, due tonon-uniform erosion.(iii) Essence exposed to waterless media erode under blocks of wood or pieces of glass, which screen that portion of essence from oxygen access. The discriminational aeration type of erosion is a localized attack on some oxygen-deficient areas, performing in characteristics localized pitting. This attack becomes more boosted with the time, because the erosion products accumulate around a small anodic area, thereby making attainability of that part more effective.

Passivity

Passivity of passivation is the " miracle in which a essence or an amalgamation exhibits a much advanced erosion-resistance than anticipated from its position in the electrochemical series ". Passivity is the result of the conformation of a largely defensive, but veritably thin(about0.0004 mm thick) and relatively unnoticeable film on the face of essence or an amalgamation, which makes it more noble. This film is undoable, non-porous and of such a " tone-mending nature " that when broken, it'll repair itself onre-exposure to oxidizing conditions. exemplifications of

unresistant essence and blends are Ti, Al, Cr, and a wide variety of pristine sword blends, containing Cr. These parade outstanding erosion resistance in oxidizing surroundings, but in reducing surroundings, they come chemically active. Grounded on trial conducted in aerated0.5 M NaCl result, the passivity of certain essence falls in the following order

Ti → Al → Cr → Be → Mo → Mg → Ni →

Co → Fe → Mn → Zn → Cd → Sn → Pb → Cu

Passivation isn't a constant state, but exists only in certain terrain conditions, which tend to maintain thin defensive oxide flicks on their shells. In the presence of oxygen, the oxide film is automatically repaired, whenever any damage occurs, but in oxygen absence, the unresistant essence and amalgamation come chemically active and are fleetly eroded. For illustration, austentic pristine brands are relatively good resistant to the action of aerated dilute sulphuric acid, but show low resistance in air-free acid. The action of further concentrated result of HNO3 on active essence(Fe and Al) produces a thin defensive oxide film, thereby stifing the anodic response and making them unresistant. This is particularly remarkable in Al, which, although veritably active chemically, isn't attacked by explosively concentrated nitric acid. On the other hand, dilute nitric acid on Fe stimulates the cathodic responses, so, rapid-fire erosion iron can do, without hydrogen gas elaboration. In case of pristine brands and titanium, the defensive oxide film is maintained, indeed in dilute HNO3, so that these parade high erosion- resistance in HNO3 result over a wide range of attention.

Pitting Corrsoion

Bending erosion is a localized accelerated attack, performing in the conformation of depressions around which the essence is fairly unattached. Therefore, bending erosion results in the conformation of perforations, recesses and depressions in the essence. Pitting is, generally, the result of the breakdown or cracking of the defensive film on a essence at specific points. This gives rise to the conformation of small anodic and large cathodic areas. In the correct terrain, this produces erosion current. Breakdown of the defensive film may be caused by(i) face roughness ornon-uniform finish,(ii) scrapes or cut edges,(iii) original straining of essence, due tonon-uniform stresses,(iv) interspersing stresses,(v) sliding under cargo,(vi) smash attack(caused by the turbulent inflow of a result over a essence face), and(vii) chemical attack. Essence owing their erosion resistance to their unresisting state, show a pronounced pitting under all conditions, which lead to the destruction of their passivity. For illustration, pristine sword and aluminum show characteristic pitting in chloride result. The presence of the extraneous contaminations (like beach, dust, scale ,etc.) bedded on the shells of essence also lead to pitting. Owing to the discrimination quantum of oxygen in contact with the essence the small part(underneath the contamination) come the anodic areas and the girding large corridor come the cathodic areas. violent erosion, thus, start just underneath the contamination. Once a small hole is formed, the rate of erosion will be increased.

Intergranular Corrosion

This type of erosion occurs along gain boundaries and only where the material, especially sensitive to sharp attack exists, and sharp liquid possesses a picky character of attacking only at the grain boundaries, but leaving the grain innards untouched or only slightly attacked. This type of erosion is due to the fact that the grain boundaries contain material, which shows electrode erosion is due to the fact than that of the grain boundaries contain material, which shows electrode eventuality more anodic than that of the grain centre in the particular corroding medium. This may be due to rush of certain composites at the grain boundaries, thereby leaving the solid essence result(just conterminous to the grain boundary) impoverished(or depleted) in one element. The impoverished solid result is andodic with respect to the grain centers as well as to the rained emulsion, so that it'll be attacked preferentially by the sharp terrain. The grain boundary type of erosion is, generally, encountered in blends. For illustration, during the welding boundary type of erosion is, generally, encountered in blends. For illustration, during the welding of pristine sword(an amalgamation of Fe, C, and Cr), chromium carbide is rained at the grain boundaries, thereby,

region just conterminous to grain boundaries becomes depleted in chromium composition and is more anodic with respect to the solid result within the grain(which is richer in chromium). For the same reason, it's also anodic to the patches of the chromium carbide so- rained. Generally, erosion un welded pristine sword isn't a serious problem, since erosion- resistance can be restored by a heat- treatment system, which dissolves the chromium carbide rained during welding. still, when pristine sword assemblies are " too large " to be heat- treated, it's also necessary to help the reduction of chromium by special means. The use of low- carbon content(about0.03) is one remedy, but generally the common0.08 carbon in sword is stabilized by the addition of titanium(as in type of 321 pristine sword). Intergranular erosion follows the path of grain boundaries and occurs on bitsy scale, without any apparent external signs of any ferocious attack. On account of this, unforeseen failure of material occurs(without anypre-warning), due to loss of cohesion between grains. The remedy is proper heat- treatment, followed by rapid-fire quenching to avoid the miscellaneous rush that, generally, occurs due to slow- cooling.

Waterline Corrosion

When water is stored in a steel tank, it is generally found that the maximum amount of corrosion takes place along a line just beneath the level of the water meniscus. The area above the waterline (highly-oxygenated) acts as the cathodic and is completely unaffected by corrosion. However, if the water is relatively free from acidity, little corrosion takes place. The problem of waterline corrosion is also that concerns marine engineers. In the case of ships this kind of corrosion is often accelerated by marine plants attaching themselves to the slides of ships. The use of special antifouling paints restricts this to some extent.

Stress Corrosion

Stress Erosion(or stress cracking) " is the concerted effect of static tensile stresses and the sharp terrain on a essence. " it is characterized by a largely localized attack being, when overall erosion is negligible. For stress erosion to do(i) Presence of tensile stress, and(ii) a specific erosion terrain are necessary. The sharp agents are largely specific and picky similar are(a) acidulous alkalis and strong nitrate result for mild sword;(b) traces of ammonia for brass;(c) acid chloride result for pristine sword. This type of erosion is seen in fabricated papers of certain blends(like high-zinc brasses and nickel brasses) due to the presence of stresses caused by heavy working like rolling, drawing or inadequate annealing. still, pure essence are fairly vulnerable to stress erosion. Stress erosion involves in a localized electrochemical erosion, being along narrow paths, forming anodic areas, with respect to the further cathodic areas at the essence face. Presence of stress produces strains, which affect in localized zones of advanced electrode eventuality. These come so chemically-active that they're attacked, indeed by a mild sharp terrain, performing in the conformation of a crack, which grows and propagates in a factory(vertical to the operating tensile stress), until failure occurs or it may stop, after progressing a finite distance. Some typical exemplifications of stress erosion are given below

1) Season cracking in a term applied to stress erosion of bobby blends, substantially brasses. Pure bobby is vulnerable to stress erosion, but presence of small quantities of alloying element(like P, As, Sb, Zn, Al, Si) affect in pronounced perceptivity. For exemplifications, nascence brass(which when largely stressed-out) suffer intergranular cracking in an atmosphere, containing traces of ammonia of amines. The attack occurs along the grain boundaries, which come more anodic with respect to the grain themselves(presumably due to complaint of tittles, caused by different exposure to conterminous grains). Both Cu,(NH3) 42 and Zn(NH3) 42 independently. This is the real cause of dissolution of brass, initiating in chink, which eventually propagates, performing in the conformation of cracks in the presence of high tensile stress.

2) acidulous embrittlement is a veritably dangerous form of stress erosion, being in mild sword exposed to alkaline results at high temperatures and stress. The failure is frequently associated with brume- boilers and heat transefer accoutrements in which water of high alkalinity attacks the mild sword plates, particularly at the crannies near riverts. Boiler – water, generally, contains a certain proportion of sodium carbonate, added for water softening purposes. In high – pressure boilers, this breaks up to give sodium hydroxide and carbon dioxide,

$$Na_2CO_3 + H_2O \longrightarrow 2NaOH + CO_2$$

and makes boiler- water alkaline. This veritably dilute alkaline boiler- water(in boiler) flows into the nanosecond hair- cracks and crannies(by capillary action), where water evaporates and the acidulous soda pop attention builds-up. This concentrated alkali dissolves iron as sodium ferroate(hypoferrite) in crannies, cracks, where the essence is stressed and the attention lf alkali is much advanced than that in the body of the liquid. The sodium ferroate (Na2FeO2) decomposes, a short distance down from its point of conformation, according to their of the following

$$3Na_2FeO_2 + 4H_2O \longrightarrow 6NaOH + Fe_3O_4 + H_2$$

$$6Na_2FeO_2 + 6H_2O + O_2 \longrightarrow 12NaOH + 2Fe_2O_4$$

response Sodium hydroxide is regenerated magnetite (Fe_3O_4) is precipitated, thereby enhancing future dissolution of iron. The iron surrounded by the dilute NaOH (main body) is the cathodic side; while the iron in contact with rather concentrated caustic soda (e.g., crevices, hair-cracks, rivets) is the anodic portion, undergoing corrosion and is thus dissolved.

Prevention of Caustic Ebrittlement

The best is addition of sodium sulphate to the boiler-water. Another is to use tannin or lignin as additive to the boiler-water. Both these methods prevent caustic cracking by blocking up the hair-cracks and crevices with innocuous harmless substances, thereby preventing the sodium hydroxide from infiltrating.

Galvanic series

In the electrochemical series(reduction electrode implicit arranged down in an adding order), a essence high in the series is more anodic and undergoes erosion briskly than the essence below it. For illustration, Li corrodes faster than Ag and so on. still, some exceptions to this conception are known. For illustration, Ti(above Ag in the electrochemical series) is less reactive than Ag. In Zn- Al couple, Zn(below Al in the electrochemical series) is eroded; while Al acts cathodic and is defended. These compliances, exactly contrary to that prognosticated by the emf series, are due to the fact that essence like Ti and Al develop, explosively clinging oxide layers on their shells, thereby making their effective electrode eventuality more positive(or lower negative).From the below, it's clear that electrochemical series doesn't regard for the erosion of all essence and blends. Accordingly, a more practical series, called galvanic series have been prepared by studying the erosion of essence and blends in a given terrain like- well water. Therefore, galvanic series give real and useful in conformations for studying the erosion of essence and blends.

Factors Influencing Corrosion:

The rate and extent of corrosion, depends on the following factors: Nature of the metal

Electrochemical Series Versus Galvanic Series

Position of galvanic series When two essence or blends are in electrical contact, in the presence of an electrolyte, the more active essence(or advanced up in the series) suffers erosion. The rate and inflexibility of erosion, depends upon the difference in their positions, and lesser is the difference, the faster is the erosion of the anodic essence/ amalgamation.

Overvoltage When a essence, which enthralled a high position in galvanic series(say zinc), is placed in 1N. H_2SO_4 it undergoes erosion forming a film and evolving hydrogen gas, the initital rate of response is relatively slow, because of high overvoltage(= 0.70 V) of zinc essence, which reduces the effective electrode eventuality to a small value. still, if a many drops of bobby sulphate ($CuSO_4$) are added, the erosion rate of zinc is accelerated, because some bobby gets deposited on the zinc essence, forming nanosecond cathodes, where the hydrogen in overvoltage of the eroding essence/ amalgamation accelerates the erosion rate.

Relative areas of the anodic and cathodic corridor When two different essence or blends are in contact, the erosion of the anodic part is directly commensurable to the rate of areas of the cathodic part and the anodic part. erosion is more rapid-fire and severe, and largely localized, if the anodic area is small(e.g., a small sword pipe fitted in a large bobby tank), because the current viscosity at a lower anodic area is much lesser, and the demand for electrons(by the large cathodic areas) can be met by lower anodic areas only by witnessing " erosion more hastily ". chastity of essence contamination in a essence generally, beget " diversity ", and form nanosecond/ bitsy electrochemical cells(at the exposed corridor), and the anodic corridor get eroded. For illustration, zinc essence containing contamination(similar as Pb, or Fe) undergoes erosion of zinc, due to the conformation of original electrochemical cells. The rate and extent of erosion increases with the adding exposure and extent of the contaminations accordingly, erosion resistance of a essence may be bettered by adding its chastity.

Physical state of essence The rate of erosion is told by physical state of the essence(similar as grain size, exposure of chargers, stress,etc.). The lower the grain- size of a essence or amalgamation, the lesser will be its solubility and hence, lesser will be its erosion. also, areas under stress, indeed in a pure essence, tend to be anodic and erosion takes place at these areas. Nature of face film In aerated atmosphere, virtually all essence get covered with a thin face film(consistence = a many Angetroms) of essence oxide. The rate of the volumes of the essence, is known as a " specific volume rate ". Greater the specific volume rate, lower is the oxidation erosion rate. The specific volume rates of Ni, Cr and W are1.6,2.0 and3.6 independently. Accordingly, the rate of oxidation of tungsten is least, indeed at elevated temperatures.

Passive character of essence Essence like Tl, Al, Cr, Mg, Ni, and Co are unresistant and they parade much advanced erosion- resistance than anticipated from their positions in galvanic series due to the conformation of largely defensive, but veritably thin film(of oxide) on the essence or amalgamation face. also, the film is of such a " tone-mending " nature, if broken, repairs itself, onre-exposure to oxidizing conditions. therefore, erosion- resistance of pristine sword is due to passivating character of chromium present in it. Solubility of erosion products In electrochemical erosion, if the erosion product is answerable in the eroding medium, also erosion proceeds at a faster rate. On the negative, if the erosion product is undoable in the medium or it interacts with the medium to form another undoable product(e.g., $PbSO_4$ conformation in case of Pb in H_2SO_4 medium), also the erosion product functions as physical hedge, thereby suppressing farther erosion. Volatility of erosion products If the erosion product is unpredictable, it volatilizes as soon as it's formed, thereby leaving to inordinate erosion. For illustration, molybdenum oxide(MoO_3), the oxidation erosion product of molybdenum, is unpredictable.

MATERIALS AND METHODS

The aim of the present study is To evaluate the inhibition of Homo Alanine in controlling the corrosion of carbon steel in the absence and presence of Zn2+.

Experimental procedure

Carbon steel specimens (0.0267%s, 0.067%P, 0.4%Mn, 0.1%C and the rest ion) of the dimensions 10.cm X 4.0cm X 0.2cm were polished to mirror finish and degreased with acetone and used for weight loss method an surface examination studies.

Weight loss study

Applicable data on the well-conditioned water used in the study are given in Table 1. Carbon sword samples, were immersed in 100 ml water and colorful attention of Homo Alanine in the presence and absence of Zn2 for a period of one day. The erosion products were gutted with Clarke's result.(32) The weight of the samples ahead and after absorption was determined using Shimadzu balance AY62. The erosion inhibition effectiveness was calculated with equation

Where,
Mdd is milligram per dm2 per day . The erosion inhibition effectiveness of(IE) was calculated using the formula
IE = W0- W1/ W0x100 Where,
W0 = erosion rate in the absence of the asset
W1 = erosion rate in the presence of the inhibitior

Analysis of the Results Of Gravimetric Studies

[HOMO ALANINE + Zn^{2+}+ WELLWATER]

The values of corrosion rate and percentage inhibition efficiency were calculated from weight loss method at different concentrations of Homo Alanine in aqueous solution that is well water after 3 days immersion period at room temperature. It was observed that Homo Alanine inhibits the corrosion of carbon steel in well water at various concentrations used in study. It is evident from addition of 50 ppm to 300 ppm of Homo Alanine. The maximum inhibition efficiency 76 % was shown at 300 ppm concentration of Homo Alanine in the absence of Zn^{2+} and further increasing inhibitor concentration does not change IE%. Indeed, corrosion rate values of carbon steel decreases from 0.1066 (mmpy) to 0.0766 (mmpy) on the addition of 50 ppm to 300 ppm of Homo Alanine in the absence of Zn^{2} ions. When the concentration of Zn^{2+}ions increases from 10ppm-20ppm to 30 ppm the inhibition efficiency slightly increases. The maximum inhibition efficiency 98% was shown at 300 ppm concentration of Homo Alanine and 30 ppm of Zn^{2+}ions and corrosion rate values of carbon steel decreases from 0.16 (mmpy) to 0.0033 (mmpy) The increased inhibition efficiency (IE%) and decreased corrosion rate might be due to the result of increased adsorption and increased coverage of Homo Alanine on the carbon steel surface with increasing concentration Homo Alanine. It is clear that Homo Alanine showed good inhibition for carbon steel corrosion in well water solutions because has nitrogen and oxygen containing functional groups.

Synergism Parameter

Synergism parameter is calculated to evaluate the synergistic effect existing between inhibitors. The synergism parameter (SI) can be calculated using the relationship given by *aramaki* and *hackerman.*

$S_I = 1-I_{1+2}/1-I'_{1+2}$

Where,

$I_{1+2} = (I_1+I_2)-(I_1 \times I_2)$

I_1 = Surface coverage of inhibitor (Homo Alanine)

I_2 = surface coverage of inhibitor (Zn^{2+})

I'_{1+2}= combined surface coverage of inhibitors (Homo Alanine) and (Zn^{2+})

IE/100 = surface coverage

When S_I>1,synergistic effect exist between the two inhibitors. In case of S_I<1,negative interaction takes place between the two inhibitors, (i.e, CR increases). The calculated synergism parameter values for Homo Alanine and Zn^{2+} synergism The results are synergism parameter (S_I) for the formulation consisting of 300 ppm of Homo Alanine and 30 ppm of Zn^{2+} ions are 8.52, which is greater than one. This shows that the synergistic effect exists between Homo Alanine and Zn^{2+}.

CONCLUSION

The synergistic inhibition of corrosion of carbon steel immersed in w e l l water has been investigated in this study. The inhibitive effect with new inhibitors viz., Homo Alanine with Zn^{2+} , Ni^{2+} , Mg^{2+} ions in controlling the corrosion of carbon steel in well water has been done. Weight loss studies for a three-day immersion period were undertaken to determine the corrosion rates and inhibition efficiencies. Potentiodynamic polarization studies and electrochemical impedance studies were carried out to determine the nature of the inhibitor formulations.

Homo Alanine- Zn^{2+} Systems

The inhibitor formulation consisting 300ppm Homo Alanine 30ppm Zn2+ ion afforded ion efficiency of 98% was achieved. Further this inhibitor combination acts as mixed inhibitor, predominately cathodic in nature. The corrosion inhibition is established due to the formation inhibiting film, provided pictorial representation on the nature of surface film formed in the absence and presence of inhibitor system. The protective film consists of [Fe^{2+}- Homo Alanine -Zn^{2+}] small amounts of iron oxide, hydroxide and zinc hydroxide.

Certain generalizations that can be drawn from this study are given below

The outcome of this work suggests that new inhibitor in combination with Zn^{2+} ion, both at low concentration developed into effective inhibitor formulations. Ternary inhibitor formulations based on the principle of antagonism will definitely be more economical and environmental friendly. The outcome of this work suggests that new inhibitor viz., Homo Alanine in combination with Ni^{2+} ion, both at low concentration developed into effective inhibitor formulations. Ternary inhibitor formulations based on the principle of synergism will definitely be more economical and environmental friendly.

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Table1: Difference between Electrochemical and Galvanic Series

S.NO	ELECTROCHEMICAL SERIES	GALVANIC SERIES
1.	Electrode potentials are measured by dipping pure metals in their salt solution of 1M concentration, without any oxide film on them	This series was developed by studying corrosion of metals and alloys in unpolluted well-water, without their oxide films, if any, removed.
2.	The position of a gives metal in electro-chemical series is fixed.	The position of a given metal may shift in galvanic series.
3.	It gives no information regarding position of alloys.	Since alloys are included in galvanic series, so their corrosion can be studied from this series

4.	The position of a metal in this series is permanently fixed.	The position of a metal, when present in the form of an alloy, is different from that of pure metal.
5.	This series comprises of metals and non-metals	This series comprises of metals and alloys
6.	It predicts the relative displacement tendencies	It predicts the relative corrosion tendencies

Table 2: corrosion rate (cr) and inhibition efficiency (ie) of carbons steel immersed in well water containing in the presence and absence of inhibitor:

Homo alanine	Zn^{2+} (0ppm)		Zn^{2+} (10ppm)		Zn^{2+} (20ppm)		Zn^{2+} (30ppm)	
	Ie%	Cr (mmpy)	Ie%	Cr (mmpy)	Ie%	Cr (mmpy)	Ie%	Cr (mmpy)
0	-	0.3266	12	0.04	17	0.27	29	0.23
50	32	0.1066	40	0.1933	45	0.1766	51	0.16
100	49	0.1666	52	0.1566	57	0.14	60	0.13
150	54	0.15	59	0.1333	64	0.1166	72	0.09
200	66	0.11	74	0.0833	80	0.0633	88	0.0366
250	71	0.0933	78	0.0733	87	0.04	96	0.0133
300	76	0.0766	89	0.033	93	0.02	98	0.0033

Table 3: Synergism parameter for homo alanine - zn^{2+} (10 ppm) system in carbon steel immersed in well water for three days:

Homo alanine	Zn^{2+} (10ppm)	I1	I2	I'1+2	Si	Ie
50	10	0.32	0.12	0.40	0.9973	40
100	10	0.49	0.12	0.52	0.935	52
150	10	0.54	0.12	0.59	0.9873	59
200	10	0.66	0.12	0.74	1.1507	74
250	10	0.71	0.12	0.78	1.16	78
300	10	0.76	0.12	0.89	1.92	89

Table 4: Synergism parameter for homo alanine - zn^{2+} (20 ppm) system in carbon steel immersed in well water for three days:

Homo alanine	Zn^{2+} (20ppm)	I1	I2	I'1+2	Si	Ie
50	20	0.32	0.17	0.45	1.0261	45
100	20	0.49	0.17	0.57	0.9844	57
150	20	0.54	0.17	0.64	1.0605	64
200	20	0.66	0.17	0.80	1.411	80
250	20	0.71	0.17	0.87	1.8515	87
300	20	0.76	0.17	0.93	2.8457	93

Table 5: Synergism parameter for homo alanine - zn^{2+} (30 ppm) system in carbon steel immersed in well water for three days:

Homo alanine	Zn^{2+} (30ppm)	I1	I2	I'1+2	Si	Ie
50	30	0.32	0.29	0.51	0.9853	51
100	30	0.49	0.29	0.60	0.90525	60
150	30	0.54	0.29	0.72	1.1664	72
200	30	0.66	0.29	0.88	2.0116	88
250	30	0.71	0.29	0.96	5.1475	96
300	30	0.76	0.29	0.98	8.52	98

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RESEARCH ARTICLE

Isolation, Screening and Characterization of Actinomycetes for L-Asparaginase Production from Rhizosphere Soil of *Caryota urens*

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ABSTRACT

L-asparaginase (L-asparagine amido hydrolase, E.C.3.5.1.1) is an effective therapeutic agent, has received considerable attention since it is used as an anticancer agent. This study attempted the isolation and screening of an anticancer enzyme L-asparaginase from actinomycetes isolated from the Maroli sugar factory, Gujarat, India. 14 actinomycete strains were isolated from the rhizosphere soil sample of *Caryota urens* using starch casein agar, actinomycete isolation agar, glycerol asparagines agar and Kuster agar. The isolated strains were screened for L-asparaginase production by the qualitative (rapid plate) and quantitative methods. All strains show L-asparaginase production on M9 medium and maximum activity obtained with AIA 1 strain in submerge fermentation (436U/ml). The most potent isolate was characterized by morphological, biochemical and molecular characterization methods. The most potent strain for L-asparaginase production was identified as *streptomyces sp.* by the 16srDNA sequencing method.

Keywords: *Caryota urens*, Soil, actinomycetes, Characterization, Screening, L-asparaginase.

INTRODUCTION

L-Asparaginases have remained an interesting research topic since their discovery 120 years ago, especially after their introduction in the 1960s as very efficient anti-leukemic drugs. Approximately, 40% of global enzyme sales are L-asparaginase, which is considered as one of the major important biomedical and biotechnological groups of therapeutic enzymes [1]. L-asparaginase (L-asparagine amidohydrolase E.C.3.5.1.1) is an effective chemotherapeutic agent to treat acute lymphoblastic leukemia and other hematopoietic malignancies [2, 3]. L-asparaginase catalyzes the

hydrolysis of essential amino acid, L-asparagine to L-aspartic acid and ammonia. The leukemic cells are dependent on exogenous supply of L-asparagine for their growth and survival as they lack L-asparagine synthetase activity. However, normal cells can synthesize their own L-asparagine, and thus, remain unaffected by its rapid depletion Caused by extraneous L-asparaginase. The L-asparaginase action leaves leukemic cells To starved for L-asparagine and induce inhibition of protein synthesis in leukemia cells [4]. It can hydrolyze both asparagines and glutamine.L-asparaginase activity was extensively reported in plants, animals and microorganisms (bacteria, fungi and actinomycetes) and also in the serum of certain rodents but not isolated from humans [5]. Isolation of asparaginase is easy from microbes rather than other sources. The production of L-asparaginase has been studied in many microorganisms such as: *P. aeruginosa* 50071 [5], *B. subtilis* WB600 [6], *Serratia marcescens* SB08 [7], *E. coli* [8], *E. carotovora* [9], *Streptomyces ginsengisoli* [10], *S. griseus* [11], *S. karnatakensis, S. venezuelae* [12], *S. albidoflavus* [13], *S. gulbargensis* [14]. The enzymes isolated from *E.coli* and *Erwinia carotovora* are now being used in the treatment of acute lymphoblastic leukemia [15]. However, these enzymes have drawbacks in that they exhibit low substrate specificity and high glutaminase activity [16]. Therefore, it is important to identify sources and methods of producing greater amounts of Asparaginase and exhibit high substrate affinity and therapeutic activity [17, 18].Actinomycetes found a new source for production of L-asparaginase. Actinomycetes are ubiquitous, slow-growing, filamentous, sporulating bacteria having high GC content. Actinomycetes have been reported to produce bioactive molecules like antibiotics, immunosuppressive agents, cosmetics, vitamins, herbicides, pesticides, and enzymes [19]. The rhizosphere soil of plants may be an attractive source of actinomycetes, capable of producing novel active metabolites. *Caryota urens* L (family: Arecaceae) is underutilized multipurpose palm species. It is unexplored in the field of microbiology. Although L-asparaginase from bacteria has been extensively characterized, more attention has been paid to actinomycetes. Taking into considerationthe current study commences for the isolation and screening of an anti-cancer enzyme L-asparaginase from actinomycetes isolated from *Caryota urens*.

MATERIAL AND METHODS

Sample collection

The soil sample was collected from the rhizosphere region of *Caryota urens* plant located at Maroli sugar factory, Navsari district, Gujarat, India. The sample was collected from up to 10-15cm in depth after the removal of ~3.0 cm of the soil from the surface. The soil sample was collected in sterile zip lock polythene bags using a sterile spatula for further work. All chemicals, media, media components, and other reagents were purchased from Hi-Media Laboratories (India) etc.

Pre-treatment of Soil Samples and Isolation of actinomycetes

The rhizosphere soil sample of *Caryota urens* plant was air-dried for 4 days and sun-dried for 3 days. Once dried, the sample was ground in pestle and mortar and sieved to exclude large particles followed by selective pretreatment methods to isolate actinomycetes by inhibiting/eliminating unwanted microorganisms. Several physical and chemical pretreatment methods have been used for the isolation of actinomycetes are wet heat, phenol and $Caco_3$ pretreatment methods. Thereafter, soil samples were inoculated on actinomycetes isolation agar (AIA), starch casein agar, glycerol asparagines agar, glucose asparagines agar and Kuster agar having antibiotics ampicillin and nystatin (50µg/ml). The plates were incubated at 35-37^{o}c for 7-15days. Colonies with suspected actinomycete morphology were subcultured, purified and stored at -20^{o}c in glycerol.

Qualitative screening for L-asparaginase and L-glutaminase production

Primary screening is a qualitative method to screen out asparaginase production of isolated actinomycete strains. Screening of potential L-asparaginase producing actinomycetes was carried out with the use of M9 medium (Na_2HPO_4 6.0g; KH_2PO_4 3.0g; NaCl 0.5g; $MgSO_4$ 2.0g; KCl 0.5g; glucose 3.0g; L-asparagine 10.0g; agar 20.0g and 1000 ml distilled water), pH was adjustedto 6.8 and supplemented with phenol red (prepared in ethanol) as a pH indicator (2.5%) and L-asparagine act as source of carbon, nitrogen and also act as substrate. Phenol red at acidic pH is yellow and at alkaline pH turns pink, thus a pink zone is formed around microbial colonies producing L-asparaginase.

Inoculated plates were incubated at 35°C for 7 days. Plates were examined for change in color of medium from yellowish to pink due to change of pH indicating the positive asparaginase activity. Colonies with pink zones were considered as L-asparaginase-producing strains. For, L-glutaminase production, L-glutamine used instead of L-asparagine as a substrate [21].

Secondary screening for L-asparaginase and L-glutaminase production

The secondary screening of L asparaginase was carried out by submerged fermentation with the isolates that showed the best enzymatic activity during the primary screening. The potent actinomycetes strain was inoculated into a 250 ml of Erlenmeyer flask containing 50ml of ADS (Asparagine dextrose broth) medium. 5% inoculums were added to the medium and flasks were placed in an incubating orbital shaker at 150 rpm at 35ºC for 5days. Medium was filtered through whatman filter paper no.1 and centrifuged at 8000rpm for 20min. The culture supernatant was used as crude enzyme source to determine the enzyme activity and protein assay.

L-asparaginase assay

L-asparaginase activity was measured by the method of Imada *et al., 1973*, which is based on the determination of ammonia liberated from L-asparagine by the enzyme reaction using Nessler's reagent. Reaction was started by adding 0.5 ml supernatant into 0.5 ml 0.04M L-asparagine and 0.5 ml 0.05M Tris [hydroxymethyl] aminomethane [Tris-HCL] buffer, pH 8.6 and incubated at 37 °C and continued for 30 min. The reaction was stopped by the addition of 0.5 ml of 1.5 M trichloroacetic acid [TCA]. The ammonia released in the supernatant was determined spectrophotometrically by adding 0.2 ml Nessler's reagent into tubes containing 0.1 ml supernatant and 3.7 ml distilled water and incubated at room temperature for 20 min and the absorbance was read in a UV-visible spectrophotometer at 425 nm. One unit of L-asparaginase activity is defined as that amount of the enzyme which catalyses the formation of 1 μmol of ammonia per min under the conditions of the assay [22].

Estimation of protein

Extracellular Protein content was determined by the method of Lowry *et al*., 1951 using BSA as the standard protein. Specific activity is expressed as unit enzyme activity per mg of protein [23].

Characterization of potent actinomycete strains

The most efficient isolate AIA 1 was characterized by morphological, cultural and biochemical characterization methods. The type of aerial hyphae, growth of vegetative hyphae, fragmentation pattern and spore formation and gram's–acid fast nature, were the morphological characteristics studied. The cultural characteristics studied were colony morphology, color of aerial and substrate mycelium, pigmentation, if any. The metabolic profile of the isolate was evaluated, by testing it for various biochemical tests like indole test, MR-VP test, citrate utilization test, sugar utilization test. The isolate was tentatively identified up to the genus level, which was further confirmed by the *16S* rDNA characterization method.

Molecular characterization

16S rRNA gene analysis was performed for confirmation of the strain at species level by isolation of genomic DNA, PCR amplification, 16S rRNA sequencing and phylogenetic analysis.

Genomic DNA extraction and 16S rDNA characterization

Genomic DNA from the most potential isolate AIA 1 was extracted using the BioRad genomic DNA isolation kit. Analysis of purity of the genomic DNA isolated was carried out using 1.0% agarose gel electrophoresis, using the DNA marker gene. The genomic DNA was further diluted and used as template DNA for polymerase chain reaction (PCR) amplification of *16S* rDNA from isolates. Primers used were as follows:

8F: 5'-AGAGTTTGATCCTGGCTCAG-3'
1492R: 5'-ACGGCTACCTTGTTACGACTT-3'

The amplification step was done using Eppendorf Thermal cycler for PCR-based amplification of *16S* rDNA. The PCR amplicon was further sequenced in forward and reverse DNA sequencing reaction with primers using BDT v3.1 Cycle sequencing kit using ABI 3730xl Genetic Analyzer. The 16SrDNA sequence was used to carry out BLAST with the database of NCBI gene bank data base. Phylogenetic trees reconstructions were obtained by the Neighbor joining method [24]. Evolutionary analyses were conducted in MEGA 11 [25].

RESULT AND DISCUSSION

Isolation and screening of actinomycetes

A total of 14 actinomycete strains were isolated from the rhizosphere soil sample of *the C.urens* plant and result shown in table 1. Current study involved the screening of isolated actinomycetes for the existence of industrially important enzyme (asparaginase and glutaminase) by rapid plate method. Potent strains were identified by a clear zone formation on M9 medium with 2.5% phenol red as an indicator. Phenol red dye is yellow at acidic pH and turns pink at alkaline pH; presence of pink color zone around the colonies is due to the liberation of corresponding enzyme. These amidohydrolases cleave amine groups and liberate aspartic acid and ammonia in case of L-asparaginase and glutamic acid in case of glutaminase. Ammonia liberated in the medium further reacts with water to produce NH_4OH resulting in increase in the pH of the medium. All 14 isolates showed pink zone around the colonies indicating increase in pH for asparaginase production and glutaminase activity and result shown in table 2. L-asparaginase and glutaminase producing actinomycete isolates in primary screening were further screened for secondary screening by nesslerization method. Among them, AIA 1 strain showed higher asparaginase activity 436U/ml low glutaminase activity 136U/ml indicated in table 3. Actinomycete isolates with promising asparaginase activity and low glutaminase activity were subjected to further study.Sahu*et al*. reported slightly higher enzyme activity in the range of 31.2-35.6 µg ammonia/ml/h from various species of *Streptomyces* isolated from fish gut under submerged fermentation [26]. Abdel *et al*. reported intracellular and extracellular L-asparaginase from *S. longsporusflavus* [27].

Characterization of most potential actinomycete strain

Morphological, cultural and biochemical characteristic of AIA 1 was reported and given in table 5. AIA 1 is gram-positive, noncapsulating, sporulating, and filamentous, which was tentatively identified as *streptomyces*. Colony characteristics and staining images are shown in table 4. As per *16S* rDNA characterization, AIA 1 isolate exhibited similarity with *streptomyces sp.* (unclassified)with accession number OP001983.1. Phylogenetic tree of AIA 1 strain indicated in figure 1.

CONCLUSION

From this work, it was clearly shown that rhizosphere soil samples of *Caryota urens* can provide a potential source of L-asparaginase-producing actinomycetes. However, in the future optimization of these isolates will be done to increase the potentiality to produce L-asparaginase.

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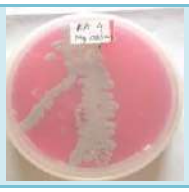
Table 1: No. of isolates on different media by pretreatment methods

Sr No.	Treatment	Media used for isolation of actinomycetes				
		GAA	SCA	KA	AIA	GLYAA
1	Wet heat	-		4		
2	Calcium carbonate	-				
3	1.5% phenol	-	1	1		1
4	No treatment (control)	-		1	6	

Table 2: Qualitative screening of actinomycete isolates for asparaginase and glutaminase production

Strains	L-asparaginase production	L-glutaminase production	Strains	L-asparaginase production	L-glutaminase production
Control					
AIA 1			KA 1		
AIA 2			KA 2		
AIA 4			KA 3		
AIA 5			KA 4		

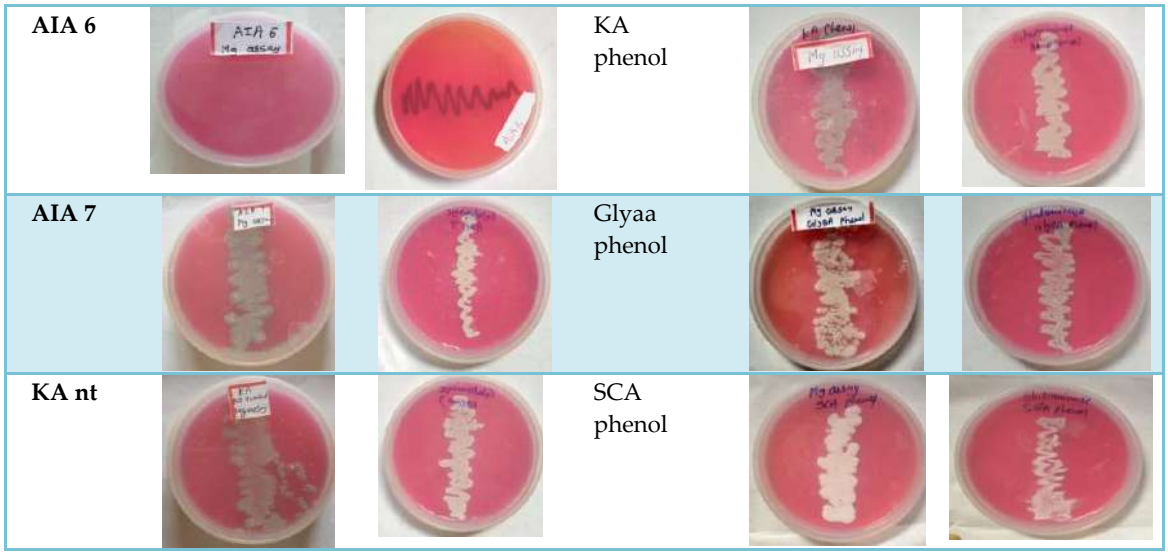


Table 3: Quantitative screening of actinomycete strains for asparaginase and glutaminase production

Strains	L-asparaginase activity (U/ml)	L-glutaminase activity(U/ml)
AIA 1	**436**	**136**
AIA 2	**419**	**228**
AIA 4	250	278
AIA 5	118	273
AIA 6	374	274
AIA 7	299	279
KA 1	122	308
KA 2	132	269
KA 3	222	251
KA 4	330	307
Kanot	230	283
KAp	175	341
SCAp	123	299
Glyaap	133	236

Table 4: Colonical characteristic and staining result of AIA 1 strain

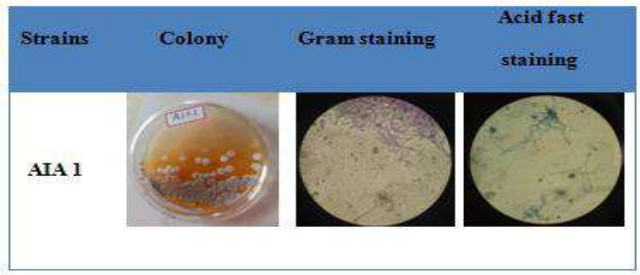


Table 5: Morphological, cultural and biochemical characterization of AIA 1 strain

Isolates	AIA 1
Aerial mycelium	+
Substrate mycelium	+
Aerial mycelium color	Grey
Substrate mycelium color	White
Pigmentation	Orange
Size	Small
Shape	Round
Color	Beige
Surface	Smooth
Elevation	Flat
Margin	Entire
Consistency	Dry
Opacity	Opaque
Indole test	-
Nitrate reduction test	++
Citrate utilization test	+
MR test	-
VP test	-
Glucose	+
Fructose	-
Sucrose	-
Lactose	-
Maltose	-
Xylose	+
Starch	-

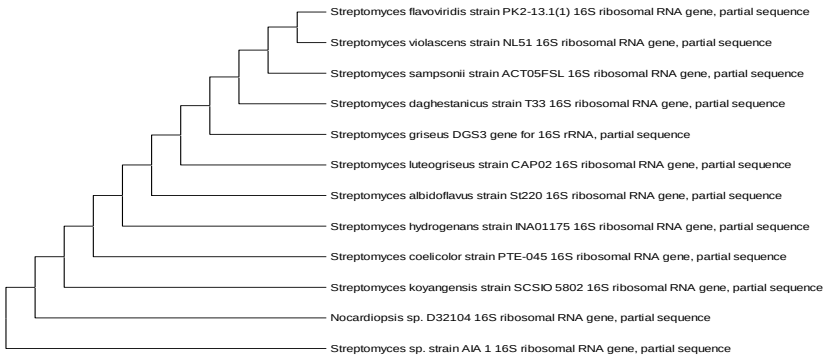


Figure 1: Construction of phylogenetic tree based on 16S rRNA gene sequencing by neighbor joining method

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REVIEW ARTICLE

Complex Generic Drugs: A Regulatory Overview

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ABSTRACT

Approval processes and regulatory standards for simple small-molecule generics have been created by regulatory bodies across the world. NBCDs, or non-biological complex drugs, lack a set of predetermined regulations in the approval process. Complex generics' regulatory frameworks are hazy and lacking in clarity. It is common for medicines to contain a large number of active ingredients, as well as molecules that are both complex and synthetic. To bring these drugs to market, it will take a more sophisticated planning and development approach, as well as a thorough awareness of the regulation, quality, and health technology assessment (HTA). The development of complex generics requires a higher level of knowledge than the development of basic generics. Complex generics need more investment than simple generic molecules, but the market for these drugs is large because they treat severe and chronic disorders. Both the industry and the FDA are facing challenges. Companies that can acclimate such significant challenges will see a substantial return on their investment. As a result, a very well approval pathway and guidance for complex generic drugs are needed. This article discusses the regulatory structures in place in the United States for bringing complex generics to market, as well as the challenges that come with developing such a drug.

Keywords: Complex Generic, Non-Biologic Complex Drugs, FDA, ANDA, Therapeutic Equivalence

INTRODUCTION

Generics have been a lucrative and appealing development trends for decades. Because the market for basic generic medications is crowded, biopharmaceutical firms are focused on complex generic treatments, which provide greater value to patients by addressing additional unmet needs while also allowing them to gain market differentiation and higher profitability. Complex generics demand a higher level of expertise than simple generics, especially in an era of rising prices and increased regulatory oversight of global innovation and manufacturing operations. It will take a more comprehensive approach to planning and development, as well as a thorough understanding of regulations, quality standards, and health technology assessment, to get these medications onto the market successfully (HTA). Generic drug companies that successfully adapt their clinical development processes to take on these new challenges while maintaining their speed to market may be eligible for both exclusivity and a significant increase in financial return. However, despite the fact that complex generics are less expensive than their brand-name counterparts, the higher risk and greater patient benefit they entail means that pharmaceutical companies can make more money off of them [1].

Generic Drugs

A generic medicine, according to the FDA, is one that is meant to be identical to a previously approved brand-name drug in terms of dosage form, safety, potency, route of administration, quality, performance characteristics, and intended use. These commonalities help to show bioequivalence, which means that a generic drug has the same therapeutic impact as a brand-name medicine. A generic drug can be used instead of a brand-name medicine[2].

Complex Generic Drug

A complex generic has a complicated active component, formulation, delivery mode, and drug device combination. Complex drugs are made up of complex active ingredients and have large, highly complex and synthetic moieties. It also includes other products for which early scientific involvement would be beneficial due to the complexity and/or uncertainty of the regulatory pathway or alternative strategies[3].

In addition to biological characteristics, NBCDs, and small molecule characteristics

NBCDs are not biologicals on the grounds that they are not acquired from living organic entities through biotechnology, yet they really do impart a few properties to natural clinical items, as displayed in Table 1.

The few examples of complex generics are: [5]

- Complex Active Pharmaceutical Ingredients (APIs) - low sub-atomic weight heparin, glatiramoids, iron sugar edifices and so on.
- Complex Formulations - parenteral microspheres, misuse obstacle generics, liposomes.
- Complex Route of Delivery - complex ophthalmological, locally acting GI drugs and skin balms.
- Complex Drug-Device Combinations - dry power inhalers, nasal showers, metered portion inhalers, and transdermal frameworks.

Background

Complex generics have been for a long time, but we've been hearing a lot more about them in recent years. This is because today's innovators are developing increasingly complex products, and the complexity of generic pharmaceuticals is growing at the same time as the complexity of Reference Listed Drugs (RLDs) [6]. In Amsterdam, an NBCD Working Group was established in 2009 with the goal of bringing together members of the pharmaceutical industry, foreign specialists, and academics to start conversations among stakeholders and raise awareness of the NBCD problem. Glatiramoids, Iron-carbohydrate medicines, low-molecular-weight heparins and liposomal were among the first products to be labelled as NBCD in 2011 [4]. It's worth noting that "NBCD" isn't a fully recognized drug product category, and the name doesn't appear in either European Union (EU) pharmaceutical legislation or US FDAregulatory materials. Instead, the FDA has used the terms "complex drug products" &"complex generics."Each

complex generic is unique in its complexity. Because of issues with physiochemical portrayal, and some of them pose substantial issues in showing pharmaceutical equivalence and for others, a basic bioequivalence research is inadequate to exhibit that they would have the equivalent clinical and as the referenced drug's safety profile (RLD). Complexity can be found in the active pharmaceutical ingredients (APIs) for several of these products, in the formulations for others, and/or in the inclusion of a drug delivery device for others [6]. In the United States, generics account for approximately 91 percent of traditional prescriptions, and complex generics are projected to capture a major portion of this market in the future. Consequently, it is critical that the medication business sees the need to change to resolve the public's issue in the United States for efficient, security, and successful conventional versions of perplexing medications. Obviously, the better they appreciate the area, the more choices they will actually want to effectively create.

Complex Generic Market Share

Generic drugs are less expensive to develop than their brand-name counterparts since they require fewer clinical trials. After the licenses and exclusivities that safeguard the marked item have lapsed, it's typical for the overwhelming majority conventional choices to arise, bringing about great market contest and lower costs [7]. In 2018, the speciality generics market in the United States was valued at US$ 18.4 billion. Currently, the growth in the industry is supported by favourable reimbursement policies, patent expiry of specialty drugs, increasing prevalence of different types of cancer, reforms implemented by government bodies and the incapability of generics to treat more complex diseases [5]. Conventional drugs are 85% less expensive than marked solutions by and large. Somewhere in the range of 2007 and 2016, conventional prescriptions saved the US medical care framework $1.6 trillion, as per the IMS Health Institute. Exactly when we contrast the amount of cures with the degree of cost, we find that undeniable things address just around 11% of all arrangements, yet they address 74% of the Medicare structure's pass-through cost, which is more than $334 billion. Generics, on the other hand, address over 89% of all arrangements anyway only 26% of the cost, suggesting a huge monetary benefit to the two patients and the medical care system.(7) In the years ahead, the market is assessed to increment at a CAGR of around 10% somewhere in the range of 2021 and 2026. As per a new report from the FDA's Office of Generic Pharmaceuticals (OGD), the FDA supported or likely cleared 948 contracted new medication applications (ANDAs) for conventional medications in 2020, including 72 first generics, which is somewhat larger number than earlier years. In 2019, the FDA supported or endorsed temporarily 1,014 ANDAs, including 107 original nonexclusive meds. As per the FDA's Competitive Generic Therapy grouping, the FDA approved 35 complex nonexclusive meds in 2020. The FDA approved 110 complex nonexclusive drugs in 2019, addressing for 11% of all conventional medication item endorsements in the year [9][10]. Small-molecule generics usually dominate the market. Since the quantity of complex generic contenders is probably going to be restricted and the obstacles to generic passage for complex items stay high, piece of the pie for complex generics is assessed to be 40%–60% [11]. Indeed, even with protection, numerous Americans-almost 60 million, as per some new buyer overviews experience issues bearing the cost of physician recommended drugs, putting their wellbeing and monetary needs in conflict. Patients and the clinical consideration structure stand to benefit altogether from complex generics. Market components and authoritative complexities, of course, are considered as basic hindrances [12].

Administrative Challenges in Producing a Complex Generic

Many companies are focusing on complex generic medication development as opportunities to foster simple generics become less appealing. Drug manufacturers can profit from complex generics if they can adjust to a more convoluted and challenging development procedure.(1) Both the industry and the FDA are facing challenges. One of the most significant challenges for the industry is those faced by the product's innovators, such as patent challenges and citizen petitions. Also, the sponsor must commit to the time-consuming and costly development of complex generics. Another challenge is the significant disparity in submission criteria among different international health authorities, making it hard to develop the same product for conveyance in various areas of the world. Also, the audit strategy utilized by the FDA's Office of Generic Drugs (OGD) for nonexclusive medication endorsements doesn't function admirably for complex generics. As a result, if a sponsor uses conventional processes toyield an abbreviated new drug application (ANDA) to OGD for a complex generic medicine, they risk being explicitly refused due to a lack of

information concerning "oneness" to the RLD or the necessity to repeat significant clinical studies [6]. Complex generic-related submissions pose a huge challenge to the OGD, in addition to the challenges faced by the industry. The OGD may find it challenging to establish clearly defined pathways for several of these products because they are so complex. In fact, the pathway for each product may differ significantly. The Generic Drug User Fee Act (GDUFA), not the least bit like its new medication partner PDUFA (Prescription Drug User Fee Act), doesn't have a plan for normal pre ANDA meeting or for accommodation of INDs, other than under incredibly one-of-a-kind circumstances. conditions. Thus, the OGD should deal with every accommodation on a singular premise [6]. The OGD, on the other hand, has communicated with the pharmaceutical industry through face-to-face meetings and controlled correspondences, and has authorized a number of complex generics. Within the GDUFA framework, the OGD is actively working to develop a framework for the review and authorization of more complex generics. Understanding and following through on the alternative submission procedures required for these products can help sponsors in overcoming or minimizing the problems associated with regulatory submissions for complex generics.

Practices to tackle these Challenges [1]

1. **Understanding administrative necessities and the objective market**: Basic generics frequently follow an obvious improvement course that remembers endpoints and data expected for FDA freedom for the United States. For convoluted generics, engineers are less inclined to get administrative direction, expanding vulnerability and chance in the preparation and configuration process. An obvious administrative methodology prompting item freedom is vital for these drives. Engineers ought to talk with controllers from the beginning to examine their improvement plan and go through their exploration plan and near examinations to guarantee they are doing great. This empowers designers to tweak their item, accelerate filings, and abstain from shocks during the advertising application evaluation.
2. **Study design and arranging:** Complex generics will quite often request more clinical examinations than basic generics, and this ought to be perceived all along. The time, region, and cost of choosing at a specific area ought to be generally considered while going with these best options, especially in the event that there is contention for patients as well as site resources, should be thought of.
3. **Site selection:** Biopharma organizations keen on making troublesome generics ought to work with firms that can help them in conquering the difficulties of finding preliminary destinations with the necessary skill, interest, and admittance to specific patient populaces. The locales best equipped for finishing these ventures are frequently reluctant to partake in generics examination, and the people who are may miss the mark on abilities expected to deal with the extra analytical requirements that muddled generics concentrates on involve.

   To mitigate the challenges, companies should seek out partners with extensive site organisation as well as the capacity and aptitude to teach novice site pioneers who wish to expand their competencies and take on more challenging projects. Since these help methods require extra education, resources, and oversight at these sites, biopharma companies should start developing site outreach programs with their partners early in the research process.
4. **competitive advantage of consistence:** Biopharma business should cautiously survey about the legitimate hardware, abilities, skill, authority, and devotion to conform to regulations, particularly on the off chance that they are under time and cost requirements. To keep away from administrative challenges that could postpone administrative leeway, organizations ought to be exhaustive in their consistence to great assembling and consistence arranging, as well as dealing with the quick timetables and complex improvement process. Because the development phase is typically rushed to expedite regulatory submission, manufacturing and QC managers claim that R&D personnel did not take proper care in creating, transferring, and verifying analytical procedures and production processes. All of these challenges pose compliance risks in any development project, but compliance issues with complicated generics projects are significantly more challenging, especially when dealing with new sites and manufacturers, or those that have just recently begun working on simple generics projects. To accomplish all efficacy, safety, and equivalence goals, complex generics development plans demand more detailed approaches and rigorous testing. They likewise face expanded compliance risk throughout the change from pilot to manufacturing, wherein processing troubles could affect batch-to-batch identity, quality, purity, potency, and consistency.

The Landscape of Complex Generic Drugs

Drug things are arranged in light of the test to evaluate drug indistinguishable quality (PE) and bioequivalence (BE) of two medicine things (i.e., the reference thing and its nonexclusive version). Orange shows standard low-sub-atomic weight sedates that can be completely characterized; exhibiting PE and BE is very clear. Biologicals are addressed by green; demonstrating PE and BE is altogether more troublesome. Blue addresses NBCDs, while white addresses other confounded medications.PE and BE are challenging to show for most of NBCDs because of the powerlessness to make homo sub-atomic material, an obscure technique for activity, as well as the trouble of completely characterizing the items. Low-sub-atomic weight heparins and egg whites bound nanoparticles are blue with a green layout (various nations characterize these prescriptions differently) [14].The chart likewise outlines a few additional intricate medications for which PE is sensibly direct to illustrate, though BE is turning out to be progressively challenging to illustrate.

Evaluating Bioequivalence in Complex Drugs [15]

The brand-name item's security and viability have recently been demonstrated; conventional medicine improvement can be more effective than brand-name drug advancement. All things being equal, the conventional medicine creator should show that they have made a chemically indistinguishable nonexclusive adaptation of the brand-name item. This infers that the conventional medication item should have the indistinguishable measurements structure as the brand name drug item, contain the identical active ingredients at the same strength, and be administered through the same route. Additionally, the generic drug product must demonstrate bioequivalence, which means that it must deliver the same quantity of the same drug to anywhere it is far wanted with inside the body the same pace and extent as the brand-name drug. In a pharmacokinetic examination, which thinks about the rate and degree to which the medication opens up in the body at different focuses subsequent to curing, one of the most exact, fragile, repeatable, and successful techniques for exhibiting bioequivalence is to analyze the potential conventional medication item and the brand name item. The FDA approves the nonexclusive medicine item as being nearly basically as protected and feasible as the brand name drug item once the review has exhibited bioequivalence and any troubles with item quality, execution, or other administrative worries have been settled.

This methodology turns out successfully for drug products that are expected to be delivered into the blood. However, establishing bioequivalence is not usually simple or proficient, especially for complex drug products. Complex prescriptions consolidate those normal to pass medicine on to neighborhood locales of activity, similar to the lungs (e.g., inhalers for asthma) or the skin (e.g., psoriasis balm), where deciding the pace of ingestion, or how much medication is available at various times, can challenge. The FDA uses a weight-of-evidence approach in this case, requiring a sequential correlation of the reference drug and the complex generic drug. When establishing the quantity of evidence required for approval of a complicated drug product, the FDA uses a case-by-case approach. The FDA, on the other hand, promotes research through the regulatory science funds provided by the Generic Drug User Fee Amendments (GDUFA)[14]. Many complex brands name drug products do not have generic drug products available due to the clinical demanding situations of establishing bioequivalence. FDA is attempting to address these issues by advancing studies that will help in the development of effective, experimentally thorough new techniques for characterizing product qualities and evaluating BE for complex drug products. This research aids the FDA in formulating industry recommendations for the development of realistic generic products. It also assists the FDA in verifying those generic medications are almost as safe and effective as brand-name medications through clinical trials. Switching to generic prescription medicines will save money for both patients and caregivers [16].

FDA Approval Pathway for Complex Generics

Low molecular weight medicines and generics are governed by the FD&C Act, whereas biologics and biosimilars are governed by the Public Health Service Act. Complex Products do not fit neatly into the FDA's current regulation frameworks for drugs, devices, biologics, or combinations of these. The FDA's Food, Drug, and Cosmetics Act applies to these complex products because they are not biologically derived. The New Drug Application (NDA) pathway is utilized to approve novel items, while the ANDA pathway is utilized to approve generics. The "conventional" nonexclusive application 505(j) (ANDA) and the 505(b)(2) application are the two truncated courses

that can be utilized to get a conventional medication item with a potential helpful identicalness rating endorsed [17]. The FDA has exhibited that the 505(b)(2) strategy can be a feasible choice for supporting confounded generics, particularly where the clinical investigations important to lay out TE lie outside the 505(j) pathway. While 505(j) applications as a rule get a remedial identicalness grade, 505(b)(2) applications don't constantly. However, the 505(b)(2) application can result in a therapeutic equivalent rating, as seen by the FDA's approval of a therapeutic equivalence rating for numerous extended-release and topical medicines. The OGD is answerable for 505(j) applications, while the Office of New Drugs (OND) is liable for 505(b)(2) applications. Additionally, 505(b)(2) considers greater adaptability as far as such examinations that can be utilized and the information and data that are obligatory to be given in application. In terms of further physicochemical characterization and/or in vivo BE investigations, only a limited degree of flexibility is allowed under 505(j). Additional clinical studies, such as analysing and comparing the safety and efficacy features of generic and RLD, may be required for the 505(b)(2) application. As a result, the 505(b)(2) application may be a more scientifically sound option for licencing complicated generics within the current regulatory framework [17].

The Obligation of GDUFA in the spread of Complex Generics

The FDA has chosen a multi-pronged procedure to speed up the improvement of troublesome generics under the Generic Drug User Fee Amendments (GDUFA) program. The FDA has ceaselessly worked on the logical reason for muddled generics starting from the presentation of GDUFA I in 2012. I laid out subsidizing for an administrative science drive under GDUFA to conquer logical boundaries that hold convoluted generics back from arriving at patients. The FDA has given proposals on logical necessities for different conventional prescription items, including complex generics, as a feature of this undertaking [18]. To date, approximately 1,900 product-specific guidance (PSGs) have been issued, with suggestions for industry to assist with accelerating the research and approval procedure in order to generate more generics, authorized, and made accessible to patients. PSGs cover a wide scope of issues, remembering complex for vitro and in vivo discharge testing. The program was a triumph, bringing about more conventional medication endorsements and higher nonexclusive medication application achievement rates.[19]

Thus, in 2017, the program (GDUFA II) was changed to widen the extent of FDA correspondence with industry. Under GDUFA II, the FDA arranged complex medication items, laid out a most optimized plan of attack audit technique for prescriptions with low contest, and made a pre-ANDA program to: [19].

- Help applicants in completing more comprehensive applications.
- Improve the efficiency and effectiveness of the ANDA review process.
- Cutting down on the number of review cycles
- Simplify the approval procedure for complicated generics

Pre-ANDA Meetings [20]

GDUFA II established the pre-ANDA meeting program to allow the FDA to provide direct feedback on ANDA filings. Applicants can ask concerns not addressed in the PSG, suggest alternate bioequivalence study plans, and get Agency opinion on complex development challenges during these meetings. Three different types of meetings that can take place:

1. **Meetings on product Development**: Take into account conversation of explicit logical issues or subjects, for example, an alternate bioequivalence approach. Permit candidates to look for FDA counsel on their well thought out course of action from an approved FDA counsellor.
2. **Pre-Submission Meetings**: Allow potential ANDA candidates to talk about and establish the format and content of their submission. Allow the FDA to highlight any concerns or data that require clarification before the application is submitted.
3. **Mid-Review Cycle Meetings**: Allow the FDA to talk with the applicant about any issues that came up during the review.

The pre-ANDA program meeting can be mentioned utilizing the CDER Direct NextGen Collaboration Portal. As a component of the system for Congress to reauthorize GDUFA III for the monetary years 2023-2027, the workplace

started the most widely recognized approach of pondering open information, meeting with accomplices, and partaking in connections with the nonexclusive medication business in July 2020.

FDA's Drug Competition Action Plan (DCAP)

DCAP was established in 2017 to encourage a strong and convenient generic medication market competition, as well as to increase the efficiency and openness of the generic drug review process without compromising scientific integrity. DCAP assists in the reduction of obstruction to generic medicine advancement and market entry, resulting in more competition and reduced consumer cost. A targeted initiative is included in DCAP to expand scientific and regulatory lucidity for complex generics. Through future innovative policies, the program's endeavours should cultivate considerably more complex generic drug development and approval [21].

International collaborations and harmonization

Convoluted generics endorsement fluctuates from one item to another, and administrative structures vary, making it challenging for industry partners to supply deep rooted accommodation information for such complex medications. Complex generics need more speculation than basic nonexclusive atoms, yet their market is significant since they fix extreme and persistent infections. As a result, precise identification and regulations for complicated generics are necessary from regulatory agencies. Harmonization of regulations is important to build up a uniform establishment for approval and to guarantee that the best prescriptions reach patients [22]. In 2020, the OGD additionally advanced harmonization endeavours. As of now, the OGD is in charge of the Generic Drug Discussion Group of the International Council for Harmonization (ICH). The GDG began its second year in June 2020 with the goal of prioritizing future potential harmonization topics, which will encompass more complex products. The first since everlastingly ICH rule (M13) that worked in orchestrating BE research plan and prerequisite for nonexclusive medications was framed in July 2020. Notwithstanding ICH responsibility, the OGD's worldwide issues division, Global Generic Drug Affairs (GGDA), worked with accomplices inside and outside the FDA to set normal worldwide advancement guidelines for nonexclusive medications. Inside the International Pharmaceutical Regulators Program (IPRP) and the Global Bioequivalence Harmonization Initiative (GBHI), the GGDA continued to lead practices in different working social occasions [22].

CONCLUSION

Complex generic medicines have a promising future in the marketplace, so the generic industry is very interested in investing in it. The market for these items is extending, and a few offices have perceived the requirement for rules around here. Complex Products, then again, have administrative and specialized section boundaries. The key impediments are showing Pharmaceutical Equivalence, Bioequivalence, and Therapeutic Equivalence. Selectiveness and an exceptional yield on speculation anticipate generics designers who can change their clinical improvement to resolve these issues while remaining on time. Complex generics, while more affordable than marked other options, furnish biopharma organizations with the potential chance to catch extra income proportionate with the extra gamble and patient advantage. The pharmaceutical sector is waiting for regulatory bodies to offer greater clarification on how complicated generics are developed and approved. For the creation of a well-established foundation of approval, it is critical that industries and regulatory bodies maintain open communication. Considering these complex products, this leads to an improvement in regulatory sciences. Patients will only have appropriate admittance to safe and financially savvy complex generics when regulatory authorities work collaboratively to identify the challenges, research required, and approval basis of complex medicines.

Conflict of Interest

The authors declare no conflict of interest.

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Table 1: StructureWell defined Complex, Heterogenous Complex, Heterogenous Manufacturing process Mostly process independent Strongly process dependent Strongly process dependent (4)

	Small molecule	NBCDs	Biological
Synthesis	Chemical synthesis	Chemical synthesis	Biological source
Molecular weight	Low molecular weight	High molecular weight	High molecular weight
Structure	Well defined	Complex, heterogenous	Complex, heterogenous
Manufacturing process	Mostly process independent	Strongly process dependent	Strongly process dependent
Stability	Stable	Partly	Unstable, sensitive to external conditions
Immunogenicity	Mostly not	Partly	Yes
Mode of action	Known	Not fully clear	Not fully clear

Table 2. Pharmaceutical Equivalence and Bioequivalence Requirements (13)

S. No.	Type of product	Demonstration of Pharmaceutical Equivalence and Bioequivalence
1.	Simple drugs	Simple
2.	Biologics	Slightly challenging
3.	Complex drugs	It's difficult to establish PE and BE for most complex drugs, such as polymeric micelles, liposomes, low molecular weight, heparins etc. Demonstrating PE is simple for other NBCDs including topical treatments, inhalers, and medications with a limited therapeutic index, but demonstrating BE is difficult.

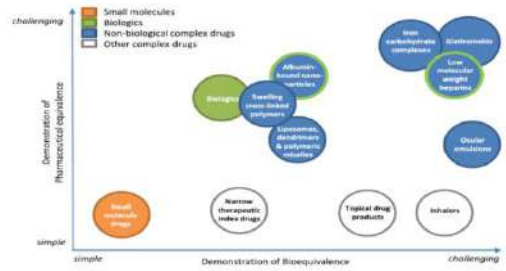

Figure 1. The complex drug landscapes. From: (Hussaarts, et al., 2017)

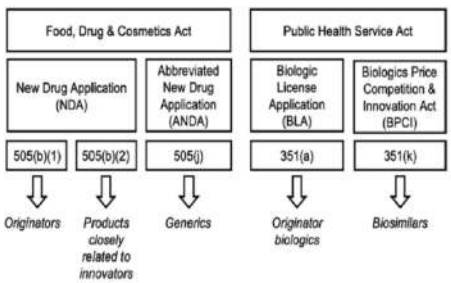

Figure 2.The FDA approval process is represented in diagram form (adapted from Hussaarts et al.).

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RESEARCH ARTICLE

Backyard Soil Vs Floor-Housed System of Rearing Indigenous Chicken – A Comparative Field Study to Ensure Nutritional Security

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ABSTRACT

Poultry is the most organised sector in animal production system. India is the fourth-largest chicken producer after China, Brazil and USA. The per capita chicken consumption has gone up from from 400 gm to 2.5 kg. To obtain maximum meat production, along with deep-litter management, backyard soil rearing of indigenous chicken is very essential. Commercially, cage system and deep-litter system are more commonly practised for raising poultry. Antibiotic residues in meat and growth promoters in broilers have also raised a major concern. In this context, Backyard soil poultry production has gained momentum. Conventionally, Backyard poultry production is an old age profession of rural families of India. It is an enterprise with low initial investment and with higher economic returns. Backyard poultry production system is a low input business and is characterized by indigenous night shelter system, scavenging system, natural hatching of chicks and poor productivity of birds, with supplementary feeding, local marketing and no health care practice. There are many advantages of rural poultry farming system one of which is enhancing the soil fertility in backyards (15 chickens produce 1-1.2 kg of manure/day). Considering these facts, the study was conducted to compare the growth rate of 12 indigenous chickens in Backyard soil and Deep litter, respectively. The growth rate of chickens in Backyard soil system was more and significantly different than deep litter system. In addition, the soil fertility was enhanced by the droppings of the chicken. Incidence of contact dermatitis and coccidiosis was less in Backyard chicken production. Recommendations were provided to promote Backyard Poultry production. Furthermore, in the villages, extension and motivational work as well as technical support should be extended to encourage farmers to rear and consume more backyard poultry as this is a means of sustainable livelihood for the poorer sections of society and will aid in food production, food security, and providing employment to rural people.

Keywords: Backyard farming, Floor-housed, Poultry, Indigenous chicken, Growth rate.

INTRODUCTION

The organized or commercial poultry sector in India contributes nearly 75% of the total meat while the unorganized sector contributes 25%. According to the 20th Livestock Census reports of the Government of India, total poultry population is 851.81 million (including backyard poultry population of 317.07 million), which is a 45.8% rise over previous livestock censuses (//www.dahd.nic.in/division/provisional-key-results-20th-livestock-census). The need for efficient food production has never been greater. one in seven humans is undernourished. Urbanization and biofuel production are reducing land availability, and climate change, lack of water and soil degradation are decreasing harvests [1]. The per capita chicken consumption has gone up from from 400 gm to 2.5 kg. To obtain maximum meat production, along with deep-litter management, backyard soil rearing of indigenous chicken is very essential. Commercially, cage system and deep-litter system are more commonly practised for raising poultry. The Supreme Court's verdict to ban cage system has raised several questions. Antibiotic residues in meat and growth promoters in broilers have also raised a major concern. In this context, Backyard soil poultry production has gained momentum. Conventionally, Backyard poultry production is an old age profession of rural families of India. It is an enterprise with low initial investment and with higher economic returns. Backyard poultry production system is a low input business and is characterized by indigenous night shelter system, scavenging system, natural hatching of chicks and poor productivity of birds, with supplementary feeding, local marketing and no health care practice. It is the most potent source for subsidiary incomes for landless and poor farmers and can easily be managed by women, children and old aged persons of the households. Backyard Soil rearing of indigenous chicken is age old practice but lost its glory in recent days after the advent of broilers. Indigenous chickens are healthy and adaptable than broilers and are much suitable for Backyard Soil rearing. Birds reared under free range conditions give eggs and meat of low cholesterol concentration compared to those produced under intensive poultry farming. Integrating livestock and crop production is one channel by which agricultural practitioners can enhance soil fertility [2]. In this context, the study was conducted to compare the two systems of rearing of indigenous chicken i.e Backyard Soil and Deep-litter method on the growth rate of chickens.

MATERIALS AND METHODS

The study was carried out in 12 indigenous breeds of chicken (Aseel breed) in both Deep-litter (Group A) and Backyard method (Group B) of rearing, respectively at Annamalai Nagar, Tamil Nadu. Visual appraisal of the appearance of the indigenous chicken types was undertaken for morphological description. The body weight of the birds was measured using a weighing scale from 1st to 18th week. Backyard rearing is as simple as rearing the birds in a fenced garden. Azolla and some house hold cereals/grains alone were fed to them. Grazing was their integral part of feeding. Deep-litter consisted of dried husk, hulls, wooden shavings, coir pith over which the birds were reared. Maize grits, Broken rice, Pearl millet, Azolla, Ragi, Soya crumbles were fed to the birds along with mineral mixture. At the end of 18 weeks, all the birds were sold off.

RESULTS AND DISCUSSION

Given some of the limitations of indigenous backyard poultry breeds, research organizations and private institutions have developed improved varieties of birds for meat, eggs or dual purpose. Improved varieties lay more eggs, gain greater body weight, have attractive plumage, involve low input costs, have high disease resistance, a better survival rate and lay large brown eggs resembling *desi* eggs. However, *desi* hens can be used for brooding eggs of improved bird varieties. In this study, Aseel breed was used to compare Backyard Vs Deep-litter system of rearing. Upto 3 weeks, all the birds were reared in Deep-litter system after which the birds included in the Backyard system were released under free range system. The body weights of the birds were presented in Tab 1.

Statistical analysis using chi-square test revealed that the growth rate was high in Backyard system than Deep litter system. However, there is no significant difference of body weight of these birds till 4 weeks of age. This might be due to the fact that up to three weeks both were raised in Deep-litter system. At the marketing age, there is significant difference between their growth rates. The rate of growth is high in Backyard soil which may be attributed to the availability of unidentified growth factors in Backyard soil. The results concurred with the observations of [3]. Apart from profitability, mortality, labour, contact dermatitis and Coccidiosis were less in Group B. They provided nutritional security to the rearers as they may not afford to buy indigenous chicken if not reared by them. Nutritional security is a requirement of rural household in developing countries [4]. The Fig 1 differentiates the advantages and disadvantages between the attributes of Backyard Vs Deep-litter (Commercial) method of rearing. It is evident that the Backyard soil rearing is rewarding than the deep-litter system. Apart from the profit, mortality, skin diseases, diarrhea due to Coccidia are less encountered in Group B.(Table 2). The close proximity among the birds and confined shelter system might be attributed to the high disease conditions and mortality in deep-litter system. The disadvantages in Backyard soil rearing is the space constraint and theft problem. It can be overcome by managemental practices. However, the productivity is not so encouraging and hence more education and support on backyard poultry keeping is essential to enhance an economic impact on the local communities [5].

CONCLUSION

Backyard poultry farming is a promising option for rural livelihoods. Lack of technical knowledge, lack of suitable germplasm, decrease in the availability of natural resources of feed and inadequate veterinary support is the alarming constraints of the traditional backyard poultry production system It requires low initial investment. It is a wise choice to boost up family income for better utilization of family laborers. While products of backyard poultry are in great demand in India, they require the right market. Community-based approaches like Self Help Groups (SHG), Farmer Producer Organizations and poultry cooperatives can provide the right platform to market the birds without the involvement of middlemen.

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Table 1 Body weight of birds at different ages in Deep-litter and Backyard soil (n=12)

Body Wt (Gms) / Age	Group A Deep-litter	Group B Backyard Soil
DO	31.5±1.52	NA
1st week	48±2.32	NA
2nd week	87±6.64	NA
3rd week	112±4.71	NA
4th week	218±4.53	225±4.62
6th week	289±5.55	300±1.78
8th week	413±9.56	450±4.98
10th week	530±8.54	700±3.67
12th week	684±7.56	780±5.76
14th week	808±7.58	900±8.45
16th week	900±4.58	1020±8.54
17th week	1010±1.57	1240±6.57
18th week	1100^{a}±2.58	1320^{b}±4.82

BW-DO is body weight at Day Old and NA is not available. The superscripts (a and b) vary significantly (P≤0.05)

Tab 2 :Comparison of different parameters between Backyard Vs Deep-litter (Commercial) method of rearing:

Parameters	Group A	Group B
Mortality	2 (16.66%)	1 (8.33%)
Contact dermatitis	6 (50%)	0 (0%)
Coccidiosis	4 (33.33%)	2 (16.66 %)
Soil Fertility	NA	Enhanced

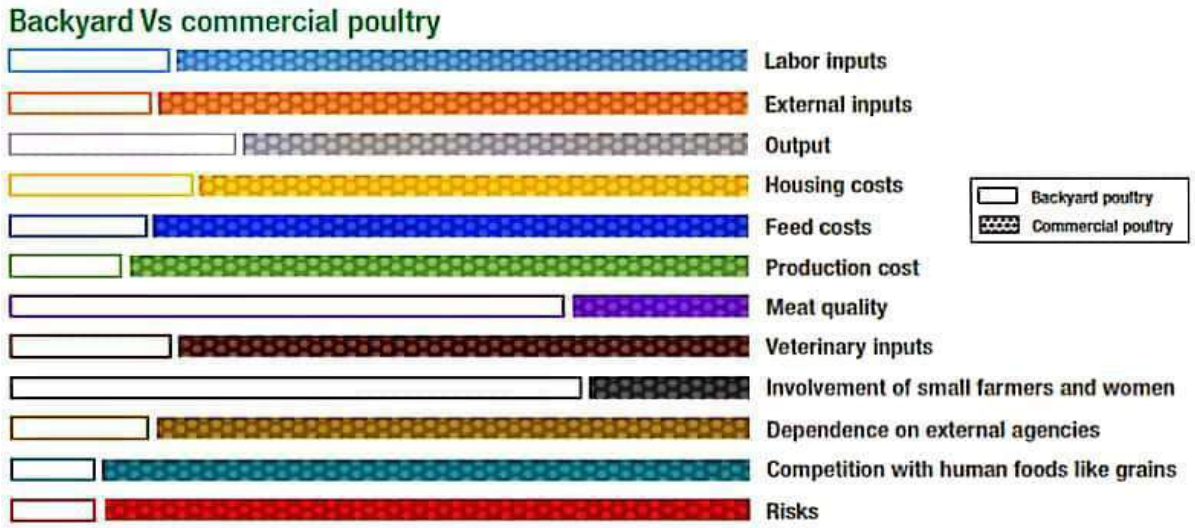

Fig: 1 Comparison of Backyard Vs Deep-litter (Commercial) method of rearing

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RESEARCH ARTICLE

Leguminous Plants Leaves: an Alternative Feed for Rohu, *Labeo rohita*

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ABSTRACT

The growth performance and approximate general composition of Rohu fish (*Labeo rohita*) fed with leguminous leaf meals were studied for 90 days duration. Two almost iso nitrogenous (25% CP) diets were formulated using groundnut (*Arachis hypogea*) and arahar (*Cajanas cajan*) leaf as the key ingredient. Fish cultured with feed without any leguminous leaf treated as a control (CN). Weight gain, Crude protein, lipid and ash content was highest ($P < 0.05$) in GF fed treatment and differed significantly with AF as well CF. Moisture content was significantly lower in fish fed with GF feed. Fish growth and other growth performance parameters are affected positively when fed GF which is good for the quantity and quality of the fish production. Groundnut leaf meal contains such ingredients which improve the growth, growth performance parameters as well as approximate general composition of fish.

Keywords: Growth performance, leguminous, isonitrogenous, groundnut and crude protein

INTRODUCTION

Fish is the one of the most important protein source in our country like India. The demand of fish is ever increasing. To make up the demand production of fish should be increased. Feed is the most important factor for fish production which occupies 50-60% of the total expenditure (Jeyaprakashsabari and Aanand, 2022). As the cost of fish meal is increasing so the cost of fish feed is also increasing rapidly. So alternative protein sources except fish meal is very much in need (Gangadhar, 2017). Animal protein sources are more costly than plant sources. Utilization of plant protein sources replacing animal sources protein reduce production cost and increase profit to some extent (Ghosh and Mandal, 2015)(Munguti et al., 2006). In case of Cotton Seed Cake up to 30%, and Sunflower seed meal up to 50% can replace ground nut oil cake in Rohu feed without any adverse effects (Dharmakar, 2021). Incorporation of plant protein is somehow tricky due to presence of antinutritional factors such as alkaloid, glycosides, oxalic acids, haematoglutinin and fatty acids (Abowei et al., 2011). Different techniques like soaked in water, dried and ground to small particle size were used before incorporation in fish feed (Lochmann et al., 2018). On the other hand, if the fish

farmers can produce an alternative feed for their own fish farm they may be able to provide healthy and hygienic feed on a regular basis. Such feed would be fresh, almost free from any pathogen and harmful chemicals. The price of feed is comparatively low than the traditional market feed. *Groundnut* and *arhar*, belong to the legume "bean" family (Fabaceae). These are cultivated throughout India as well as West Bengal. After harvesting huge amount of plant residue is left in the crop field which contains a significant amount (20- 23%) of crude protein (Table 1). Many studies have been carried out to evaluate the effects of nonconventional ingredients used in diets as fish meal (FM) substitutes on fish (Bag et al., 2011). As part of investigations examining the suitability of groundnut and *arhar* leaf in diets for Rohu (*Labeo rohita)* the main objective of this study was to evaluate growth, proximate composition of fish flesh in response to the alternative feeds.

MATERIALS AND METHODS

Twenty fingerlings of Rohu fish in triplicate groups used in three different treatments. The fish fingerlings were treated with potassium permanganate solution (1 mg L–1) to remove any external parasites and were acclimatized in a big tank for three days. The experiment was conducted for 90 days from June to August in the year 2021 at the tanks of 1000 litres capacity cemented rectangular tank. In each tank the bottom is filled with some inert soil. The water was exchanged in all the tanks at 7 days interval. A constant depth of water was maintained adding water at 3 days interval. The principal feed ingredients were collected from local agricultural field which contained significant amount of crude protein (about 20%). These substances were procured at minimum cost. Biochemical compositions of groundnut and arhar leaf used for feed for tilapia are shown in Table 1. Diets used for growth trial were prepared that feed formulations remain Almost isonitrogenous (25 g 100 g–1) and isoenergetic (4.0 Kcal g–1) in nature. Details of diet formulations are presented in Table 2. Mustard oil cake, wheat flour and egg shell dust were common ingredient in every feed tested. These ingredients were used to compensate lipid, protein and ash deficiency in formulated feed. Each feed was fortified with egg shell dust which is available Almost free of cost for calcium supplementation. This was added keeping in mind that the developing fish needs huge quantity of calcium for its bone development. The different ingredients were thoroughly mixed using a food mixer (A200 Hobart Ltd). The proportion of different feed ingredients was determined by using Pearson's square method. The mixture was given the shape of pellets using a Pellet Mill (Model CL2) with a 12 mm die. The resulting pellets were dried in a hot air oven for 48 h at 50 ºC and then packed in polythene bags for frozen. The feed was given ad libitum in a feeding bag hung from an iron rod in four locations in each tank. Unconsumed feed was removed after 1hour from the beginning of feed administration. Growth and nutrient utilization were determined in terms of feed intake (FI), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and hepatosomatic index (HSI) as follows: (Bag et al., 2011).

FI (g fish-1 day-1) = total feed intake per fish/number of days
SGR (% day-1) = 100 × (ln[final body weight]-ln[initial body weight])/no. of Days
FCR = feed intake/live weight gain
PER = live weight gain/crude protein intake
HSI (%) = 100 × (weight of liver/total body weight)
GSI (%) =100 × (weight of gonad/total body weight)

Feeds and carcass samples were analyzed following standard procedures (AOAC, 2000): dry matter (DM) after drying in a hot air oven (Gallenkamp, UK) at 105 °C for 24 h; crude protein (CP) by Kjeldahl method (N × 6.25) after acid hydrolysis, crude lipid (CL) after extraction with petroleum ether for 7-8 h by Soxhlet method (40 60 °C boiling range), total ash by igniting at 550 °C for 3 h in muffle furnace (Size 2 Gallenkamp, UK). Organic matter (OM) was calculated by subtracting total ash from DM (Giri et al., 2000). Crude fibre was determined using a moisture free defatted sample which was digested by a weak acid HCl (0.1N) followed by a weak base NaOH (0.1N) using the Fibertec System 2021 (FOSS, Denmark). Nitrogen-free extract was determined by subtracting the sum of crude protein, crude lipid, crude fibre and ash from DM (Maynard et al., 1979).

RESULTS AND DISCUSSION

A steady and rapid growth of fish was noticed till 80 days of release of the fingerlings (irrespective of treatment variations) in the experiment (Figure 1). The growth of fish was slightly declined after 80 days to till hervest. However, the rate of growth was quite faster in case of fed fishes than that of control. In final observation i.e. on 90 days after release the weight of individual fish under GF treatment was maximum than the other two treatments (Figure 1). In the present investigation the amount feed intake (g fish-1 day-1) ranged 1.27–2.13. It was recorded lowest in control treatment (CF) (1.27 g) and maximum in AF (2.13 g). These results show an encouraging response of the fish to the newly formulated feeds. Similar finding also reported from Ogunji et al. (2008). GF exhibited superior results to AF as well as control. This may be due to the better acceptability of GF than other feed offered. The SGR was obtained maximum in GF (0.91) followed by AF (0.82) and CF (0.72). FCR, an important indicator of feed utilization efficiency was recorded lowest in GF (2.25) and highest in CF (2.73).This indicates that fish can assimilate and utilize the GF based feeds well than the other feeds. Similar observation was reported from Thompson et al., 2005on juvenile Australian red claw crayfish (*Cherax quadricarinatus).* It is indicative to the fact that the feed prepared with groundnut leaf contains some such ingredients which decrease the FCR suitable for fish culture (Ebrahim and Abouseif, 2008).

The highest PER value in the present study was recorded from GF (1.46) fed fish indicating that quality of protein as well as its richness of amino acid profile in groundnut leaf is better than the other feed ingredients. Hepatosomatic index (HSI) was measured at the end of the experiment to evaluate condition and nutritional status of fish. A significant high value of HSI (1.81) was obtained from GF for Rohu. Gonadosomatic index (GSI) is a tool for measuring the sexual maturity of animals in correlation to ovary and testis development. The GSI value of the present investigation for *Rohu* was in the range of 1.26–1.52(Table 3). Growth of animal is a complex process influenced by its genotype, hormonal status, nutrition and the environment under which it grows (Ayoola et al., 2010). In the present study it is observed that the fish fed with the diets formulated with two alternative sources had different effects on various growth parameters like body weight, body length, SGR and FCR. This might have happened possibly because of differences in acceptability and palatability of feeds and the environmental condition of the tank. Although the genetic potential for growth may differ among fish, nutritional and hormonal factors are significant contributors to the expression of that genetic potential for growth and efficiency of nutrient utilization (Ayoola et al., 2010).

CONCLUSION

The feed formulating from groundnut leaf exhibits not only maximum yield than other feeds offered but it reduces the production cost to some extent. Unused plant residual part can be incorporated by this way which reduces the labour cost and pollution. Local employment generation can take place which is the burning problem of our society.

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Table 1. Biochemical composition of Groundnut and Arhar leaf used for feed for Rohu (*Labeo rohita)*

Ingredient (%)	Groundnut leaf	Arhar leaf
DM (Dry matter)	93.67	93.32
CP (Crude protein)	22.15	19.68
CL (Crude lipid)	8.79	8.33
Carbohydrate	10.28	9.57
Ash	8.95	9.09
NFE (Nitrogen free extract)	34.79	37.09
CF (Crude fibre)	8.21	8.96
GE(Kcal g-1)	3.33	3.24

Table 2: Detailed information of formulated diet.

Name of feed	Ingredients	% of CP in ingredient	% of ingredient in formulated feed	% of crude protein in feed	% of lipid in feed	% of carbohydrate in feed
GF	G N Leaves	22.24	40	25.03	8.0	10.1
	MOC	34.54	30			
	Wheat flour	9.11	28			
	Egg shell dust	1.6	2			
AF	A leaves	20.33	42.4	24.80	8.1	10.2
	MOC	34.56	31.1			
	Wheat flour	8.97	25.4			
	Egg shell dust	1.6	1.1			
CN	MOC	35.6	38.1	24.75	8.0	10.3
	Wheat flour	9.05	60.4			
	Egg shell dust	1.6	1.8			

Table 3. Growth performance and nutrient utilization of Rohu (*Labeo rohita)*fed CF, GF and AF diets (mean±SD)

Particulars	CF	GF	AF
Initial weight (g)	20.0	20.0	20.0
Final weight (g)	85.23	120.15	114.12
Initial length (cm)	8.0	8.0	8.0
Final length (cm)	11.8	15.1	13.2
Feed intake (g fish-1 day-1)	1.27	2.13	2.08
Specific growth rate (% day-1)	0.72	0.91	0.82
Feed conversion ratio	2.73	2.25	2.57
Protein efficiency ratio	1.20	1.46	1.29
Hepatosomatic index	1.51	1.81	1.71
Gonadosomatic index	1.28	1.52	1.38

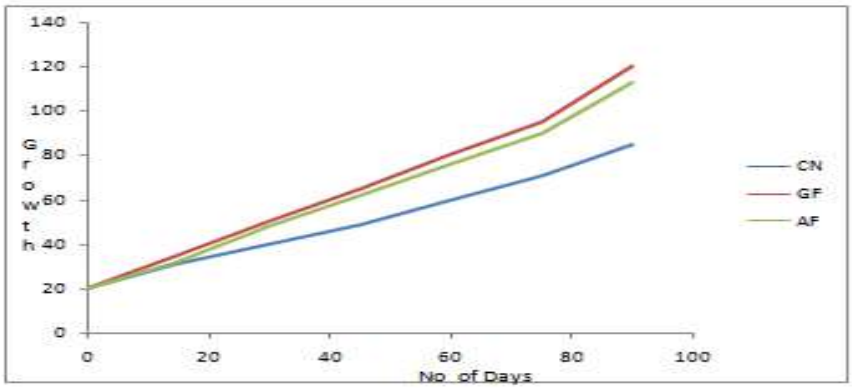

Fig 1. Growth Performance of fish fed with CF, GF and AF feed

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RESEARCH ARTICLE

Genetic Variability and Heritability Studies in Landraces of Pumpkin (*Cucurbita moschata* Duch ex. Poir)

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Tamil Nadu, India.

ABSTRACT

Investigation was conducted during 2018-19 at Vegetable unit, Department of Horticulture, Annamalai University, Annamalainagar in 20 diverse genotypes of pumpkin. The experiment was laid out in Randomized Block Design with three replications. These genotypes were used to assess the variability, heritability, genetic advance. Fourteen quantitative traits *viz.*, vine length, number of branches, days to first male and female flowering, node number of first female flower, sex ratio, days to first fruit harvest, fruit length and girth, number of fruitsplant^{-1}, average fruit weight, 100 seed weight, Total Soluble Solids, yield vine^{-1}. Data were analyzed statistically for phenotypic and genotypic variance, coefficient of variation, heritability, genetic advance and genetic gain. Phenotypic variability was found to be high for all the characters indicating their response for effective selection. It has least role of environment on these traits. High heritability was recorded for the all the fourteen characters. High heritability along with high genetic gain reveals the predominance of additive gene action of ten traits. The present study revealed that yield vine^{-1} was significantly and positively influenced by vine length, number of primary branches, sex ratio and total soluble salts. Thus, these characters constitute the selection criteria for improvement of yield vine^{-1} in pumpkin.

Keywords: Variability, Heritability, Genetic advance

INTRODUCTION

Pumpkin (*Cucurbita moschata* Duch. ex Poir.) is an important vegetable that belongs to the family Cucurbitaceae having chromosome number 2n= 40. It is mainly grown for its fruit and it contains 1.4 g of protein, 0.7 mg of iron, 10 mg of calcium, 2 mg vitamin C, 30 mg of phosphorus and 50µg of carotene in 100 g of edible portion. The fruit is an excellent source of vitamin C, vitamin E, lycopene and dietary fibre. Flowers are more nutritious than fruits. In pumpkin, various parts of the plant are used for different purposes especially for some medical treatments. Young tender tops of shoots and leaves are also cooked as vegetable. Seeds (kernels) of pumpkin are highly nutritious and used in confectionary. The leaves are haematinic, analgesic and are also used externally as treatment for burns. The pulp is used to relieve intestinal inflammation or enteritis, dyspepsia, diuretic and stomach disorder and used to reduce tapeworm infection. Pumpkin is a large, showy, yellow flowered, monoceious, highly pollinated, entamophilous species in the cucurbitaeae. In Tamil Nadu, maximum diversity is found for its fruit shape, fruit colour, vine length and yield characters. There are different types of fruit shape in pumpkin *viz.,* elongate, oblong, globular, elliptical, cylindrical, etc. The fruit colour varies from yellow to orange. The fruit skin ranges from smooth to slightly ribbed skin. Among the cucurbitaceous vegetables, pumpkin has a place of high value due to its long shelf life, long period of availability, excellent response to vegetable forcing, high nutritive estimates and better transport qualities. Though, there is a wide range of genetic variability available in India, not much attention has been given to the genetical improvement. The success of any breeding programme mainly depends upon the extent and magnitude of variability existing in the germplasm. Knowledge of the estimates of genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance would help in selecting appropriate breeding methods. Keeping in view the above facts, the present investigation was undertaken in pumpkin with the objectives to study the relative magnitude of genetic variability, heritability and genetic advance in pumpkin.

MATERIALS AND METHODS

The experiment was conducted at the Vegetable Unit, Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Cuddalore, Tamil Nadu, India. Twenty pumpkin germplasm were collected from different districts of Tamil Nadu for the experiment. The experiment was laid out in a randomized block design with three replications of each genotype. Pits of 60 cm diameter and 30 cm depth were taken at a spacing of 2 × 1.5 m. In each pit, five seeds were sown. Sowing was done in such a way that in each replication there was a single row of two plants per accession. The cultural and management practices were adopted according to the package of practices recommended by Tamil nadu Agricultural University. Five plants in each accession were tagged for recording the biometrical observations. The mean value of five plants in each genotype and in each replication was subjected to statistical analysis of variance table. The estimation of mean, variances and standard error were worked out by adopting standard methods [14]. Genetic parameters like variance, genotypic coefficient of variation, phenotypic coefficient of variation, heritability and genetic advance were calculated [6] and [7].

RESULTS AND DISCUSSION

Selection is effective only when there is wide genetic variability among the individuals in a population. Phenotypic and Genotypic variance ranged from 0.99 to 325.95 and 0.99 to 326.01 were recorded respectively. Phenotypic coefficient of variation (PCV) was slightly higher than the genotypic coefficient of variation (GCV) for all the characters under study (Table.1). This indicates that the environmental influence is very low. Hence, selection for these characters would be made based on their genotypic performance. High GCV values for most of the characters revealed the presence of high magnitude of genetic variability in the population studied. Very low ECV was recorded for all the fourteen characters. It indicated that low influence of environment of variability expression of these traits. The extent of genetic variability observed in the present study for the most of the attributes in pumpkin

was quite high and the same can be exploited by the breeders for increasing the productivity. [15]. Greater magnitude of PCV and GCV was observed for sex ratio, fruit length, fruit girth, average fruit weight, number of fruits vine^{-1}, 100 seed weight, total soluble solids (°Brix) and yield vine^{-1}.These results coincide with those of[21], [9] and [1]. Very high magnitude of PCV and GCV for these traits indicates presence of very high degree of variability and better scope for improvement. Moderate GCV and PCV for vine length (m), number of primary branches and node number of first female flower indicated the existence of comparatively high variability, which could be exploited for improvement through selection in advanced generations. These results were in conformity with those of[8] and [13].The characters like days to first male flowering, days to first female flowering and days to first fruit harvest recorded low PCV and GCV values. It indicates the presence of narrow genetic base for these traits. The estimates of these parameters are in line with the findings of[21], [9]. Improvement in these characters can be brought out by hybridisation to widen genetic base and then selecting in advanced generations. Existence of mere variability in the population may not serve the whole objective of breeding programme. Therefore, it is essential to partition the overall variability into its heritable and non-heritable components for predicting the genetic advance, which will enhance the precession of selection. The heritability is a good index of the transmission of characters from parents to their offspring's. Selection will be effective when the heritable estimates for the different characters are high. High genotypic coefficient of variation is not sufficient for determination of the heritable variation, as it simply measures the extent of genetic variability present for a character. Hence, GCV together with heritability estimates would give the best picture of the extent of advance to be expected by selection. Improvement in mean genotypic value over the base population as per cent of mean is known as genetic advance as per cent of mean.

It depends on the heritability of the trait, the genetic variability in the base population and the selection intensity. Hence, it is essential to combine both heritability and genetic advance as per cent of mean together with GCV will give a clear picture to select a better parent [6]. In the present investigation, all of the characters studied had high heritability. The characters vine length, number of primary branches, days to first male flowering, days to first female flowering, node number of first female flower, sex ratio, average fruit weight, days to first fruit harvest, fruit length, fruit girth, number of fruits vine^{-1}, 100 seed weight, Total Soluble Solids and yield vine^{-1} recorded high heritability estimates above 60 per cent (Table.2). Similar high heritability estimates were previously reported by [17] for vine length, 100 seed weight and fruit girth. High heritability coupled with high genetic advance as per cent of mean were established by the traits vine length, number of primary branches, node number of first female flower, sex ratio, average fruit weight, fruit length, fruit girth, number of fruits vine^{-1}, 100 seed weight, Total Soluble Solids and yield vine^{-1}. Same trend of high heritability coupled with high genetic advance was reported for these characters by [5] and [11]. These results indicated that the characters like vine length, number of primary branches, node number of first female flower, sex ratio, average fruit weight, fruit length, fruit girth, number of fruits vine^{-1}, 100 seed weight, Total Soluble Solids and yield vine^{-1} indicated the presence of additive genes and so these traits can be improved by a simple phenotypic selection. Among the characters studied, days to first male flowering, days to first female flowering and days to first fruit harvest registered high heritability coupled with moderate genetic advance indicating the action of non-additive genes for expression of these characters indicating that these characters can be given considerable weightage by a simple phenotypic selection. A similar report on high heritability and moderate genetic advance was reported by [9], [5], [3] and [11].

CONCLUSION

The present study revealed that yield vine^{-1} was significantly and positively influenced by vine length, number of primary branches, sex ratio and total soluble salts. Thus, these characters constitute the selection criteria for improvement of yield vine^{-1} in pumpkin. High heritability was recorded for the all the fourteen characters. High heritability along with high genetic gain reveals the predominance of additive gene action of ten traits.

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Table.1. Magnitude of variability for various characters in pumpkin genotypes

S.No	Characters	PV	GV	PCV (%)	GCV (%)	ECV (%)
1.	Vine length (m)	1.04	1.03	16.76	16.63	2.09
2.	Number of primary branches	1.58	1.56	13.41	13.33	1.46
3.	Days to first male flowering	24.49	24.23	9.55	9.50	0.98
4.	Days to first female flowering	26.46	26.39	8.83	8.82	0.46
5.	Node number of first female flower	5.83	5.80	12.92	12.88	1.02
6.	Sex ratio	6.65	6.63	20.07	20.05	0.98
7.	Days to first fruit harvest	45.25	44.94	8.01	7.99	0.65
8.	Fruit length (cm)	80.90	80.83	29.66	29.65	0.88
9.	Fruit girth (cm)	325.95	326.01	32.93	32.92	0.07
10.	Average fruit weight (kg)	1.13	1.13	45.47	45.46	1.03
11.	Number of fruits vine^{-1}	3.08	3.08	38.34	38.33	0.90
12.	100 seed weight (g)	6.78	6.78	27.21	27.21	0.25
13.	TSS (°Brix)	0.99	0.99	23.99	23.98	0.62
14.	Yield vine^{-1} (kg)	3.07	3.07	20.47	20.44	1.00

Table.2. Estimation of heritability, genetic advance and genetic advance as per cent of mean for various characters in pumpkin genotypes

S.No	Characters	Heritability h^2 (%)	Genetic advance	Genetic advance as per cent of mean
1.	Vine length (m)	98.44	2.07	33.99
2.	Number of primary branches	98.80	2.56	27.30
3.	Days to first male flowering	98.93	10.08	19.47
4.	Days to first female flowering	99.72	10.56	18.15
5.	Node number of first female flower	99.37	4.94	26.45
6.	Sex ratio	99.75	5.30	41.25
7.	Days to first fruit harvest	99.31	13.76	16.40
8.	Fruit length (cm)	99.91	18.51	61.06
9.	Fruit girth (cm)	99.98	37.18	67.82
10.	Average fruit weight (kg)	99.94	2.19	93.62
11.	Number of fruits vine^{-1}	99.94	3.61	78.94
12.	100 seed weight (g)	99.99	5.34	56.05
13.	TSS (°Brix)	99.93	2.05	49.38
14.	Yield vine^{-1} (kg)	99.76	3.60	42.07

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RESEARCH ARTICLE

Challenges in Solid Waste Management in a Mountain Region: a Study of Gangtok, Sikkim

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ABSTRACT

Gangtok, the capital of Sikkim is one of the largest town in the state, located in the eastern Himalayan Region of the Indian state of Sikkim. Present paper aims to portray and enlighten the current waste management and practices in Gangtok. Reviews, the lately planned solid waste management system. To categorize the factors that influence waste management in Gangtok. The examination investigated the present strong waste administration framework and recognized the qualities and the shortcomings of the framework. It was seen that the present strong waste administration framework rehearsed in Gangtok was unfeasible. The assortment was deficient and there was no arrangement for composting of the waste. Larger part was dumped in open landfill and individuals were not engaged with strong waste administration framework. Further, it was discovered that new strong waste administration plan outlines a framework of waste segregation system. Fertilizing the soil of biodegradable waste is a significant element of the proposal. The proposed framework, be that as it may, precluded basic focuses which should be tended to build up a maintainable strong waste administration framework.

Keywords: Solid Waste Management, Mountain, Gangtok, Strong Waste Administration Framework.

INTRODUCTION

Mountains assume a significant job in elevating the travel industry and nearby entertainment. Their area in a delicate natural condition, frequently far away from other foundation, troublesome vehicle conditions and now and again outrageous climatic conditions represent a test for the stockpile with water, vitality, and merchandise and for the removal of waste. In creating nation like India, the issue related with strong waste administration and more precise

than of created countries. Due to lack of resources and basis amenities to work on the proper management of solid waste makes it a vicious cycle; lack of assets leads to poor administration arrangements, which leads to fewer people willing to pay for those administrations, further destroying the asset base. The issue is further complicated by the rapid urbanisation and population growth, which greatly increase the amount of garbage produced and the demand for waste recovery management in civil lands. But generally speaking, an increase in population is not accompanied by a corresponding increase in money for the local districts to support the board. In addition, rapid urbanisation results in the rapid creation of shanty dwellings, which are often impromptu and exacerbate the problems with trash, health, and hygiene. Another noteworthy factor that adds to the issue of strong waste in a creating nation situation is the absence of legitimate assortment and transportation offices. Ill-advised arranging combined with quick development of population and urbanization serves to include clog in roads, and accordingly the waste assortment vehicles can't arrive at such places, hence permitting rottenness to develop after some time. Absence of fiscal assets, now and again, brings about inappropriate or no transportation vehicles for waste removal adding another measurement to the consistently rising cycle of issues.

The Study Area

With a total population of 100,000 and a demographic mix that includes Nepalese, Bhutias, and Lepchas, Gangtok, the capital of Sikkim, is one of the largest cities in the state. It has a land area of 7.82 square kilometres and is located in the eastern Himalayan region of India. Due to its lovely location and enchanting atmosphere, Gangtok has become a popular destination for tourists. Gangtok has developed into the centre of Sikkim's tourism industry, attracting visitors from all over the world throughout the year.

From Tashi View Point, the limit of Gangtok extends eastward along the valley side of Easter Bye Pass Road all the way to the ManeyJhora. From this point, the limit continues downstream along ManeyJhora, along the P.H.E. Pipeline Road, along the edge and down till the principal diverting, and from there, along the Hanuman Tok link street until the second mile Check Post. Post the limit runs downhill corner to corner till the DichilingJhora from the following mile check. The boundary continues downstream along the DichiingJhora cost 300 feet below the Rongnek Road from here.[1]. India being the world second largest populated nation [1]. According to the 2011 Census of India, there were over 1.12 billion people living there, accounting for about 17.5% of the world's population and 24% of its total land area. In addition, India's urban population is expanding far more quickly than its rural population. A considerable increase in GDP causes the waste problem to worsen in addition to the population growth (Gupta et. al., 1998) [2]. Conferring to the status statement of MSW by (CPCB,2011) [3].India creates around 127.49 million tons of MSW a day, making it 6th biggest MSW producing country on the planet(Xu, T. J. et. al., 2014) [4]. Solid Waste Management in India, customarily among the metropolitan advancement is the most overlooked region which accounts the majority of ecological and medical problems since past and in the present as well, bringing about many issues connected with wellbeing, sterilization, and other natural issues. In India the elements for the unfortunate capability of strong waste administration is a direct result of absence of monetary assets, institutional shortcoming, and improper decision of innovation. As a matter of fact, in India assortment of waste is exceptionally poor in little and medium towns. Disperse trash and litter wherever in and around the urban areas and towns makes it look clogged and unfortunate, the greater part of the waste created stays dissipated causing irresistible and parasitic episode.

Prominence of Solid Waste Management

Gangtok at present have about 55.5 per cent houses in the urban population of Sikkim. This might be credited to the differences in the local advancement design because of the fast increment of population in a constrained region. According to 2001 enumeration the number of inhabitants in the town was just 29.54 per cent inside the old town region however it is exceptionally evident that the sensible town limits which is appropriate according to the GOS\4D and HD\6(70)294dated 23\11\04 is evaluated to suit around right now 140,000 to 150,000 individuals [5]. A few highlights of the quick urbanization are as per the following:

1. At present Sikkim do not have an urban body that investigates the administration of the town. The UD&HD and the PHED are the essential offices liable for the town advancement and the executives of centre municipal assistance.
2. There is an unsatisfactory usage of the structure guidelines that has prompted provincial lopsided characteristics regarding metro framework improvement.
3. Development patterns are assessed to keep focusing on the significant towns which will prompt exacerbation of the unevenness in the effectively hard squeezed metro offices.
4. From December 2005, the legislature of India has propelled a JNNURM program for the following seven years for the improvement and advancement of urban communities the board. Gangtok is one of the 63 urban communities all over India chose for the mission program. Strong waste age is straightforwardly connected to the population thickness. In small country networks with exceptionally less population the local waste produced is charmed by the nature itself through a characteristic framework [6]. The level of degradation is normally higher in well-built waste arrangement of the provincial networks. Along these lines, the issue of waste administration framework is contacted in high densities populated zones and unmanaged development of settlements in and around Gangtok. However, in the rural areas the issues of waste administration are not stroked. The regions having high population and quick urbanization the administration of waste has been a huge test. In this manner, this contextual analysis brings into the centre the earnestness of appropriate administration of strong waste in Gangtok town.

Solid Waste Output

Waste which contribute as strong waste in Gangtok incorporate household squander, attractive waste, building material waste, road waste and so on. Roughly 45 metric Ton for every day (MT\day) is created in Gangtok and its bordering regions. The significant source of waste created in Gangtok is from household waste ,administrative centre 34.19 per cent followed by business and institutional waste with 28.1 per cent and19.2 per cent separately. 3.17 per cent of waste is accounted from the agrarian segment, just 0.12 per cent and 15.22 per cent individually accounts in mechanical and other waste. Around 27,000 kg\day of all out civil waste is created in Gangtok. As indicated by the UD&HD the per capita age of strong waste in Gangtok is 0.385 kg\day [7]. Waste created from household which incorporates kitchen waste, for example, vegetable waste, metals, plastics, and bottles are the biggest givers. Other waste patrons incorporate institutional waste, waste produced from schools, universities, workplaces, banks, medical clinics, strict spots, which incorporate left over nourishments, plastics, and papers and even human excreta. Business waste from lodgings and cafés incorporate nourishment readiness waste like remains, rotted, terminated dairy items, and dismissed products. Waste from dairies, hen's ranches, steers ranches and their excreta are the loss from horticultural part. Other biodegradable waste incorporates side of the road tress leaves, branches and so forth.

Practices in Administration of Solid Waste

The correct administration of strong waste involves assortment of waste materials, and their appropriate removal is reasonable dump site and cremation. The initial step that we must take is the best possible assortment of waste followed by the executives of waste through proper, specialized, and logical treatment of the gathered waste [8].

Collection

Collection and transportation framework is set up and a Door-to-Door assortment of families provided to the waste get vans and collection of waste from the inaccessible areas by the back packers manually taken up to the road where the pickup vans collects the waste. The pickup vans are uniquely structured secured waste assortment vehicles that transport 500 to 600 kgs of waste. All pickup vans relay to the exchange station situated in an advantageous area. Tipping trucks of about 10MT burden move the loss from the exchange station to the waste administration plant found 18kms away at Martam. The state has taken every one of the end eavours for the best possible administration of waste in partnership with UD&HD division Government of Sikkim. Dustbins have been set along the side of the road at different focuses in Gangtok from which the waste is gathered. The waste is isolated into Bio-degradable and Non-biodegradable making it obligatory through city by laws for the residents or different organizations concerned. There are two packs green and red which imply degradable and non-biodegradable separately. The act of keeping

dustbins before shops and houses are obligatory and to make it progressively advantageous for the individuals even the Government has given dustbins to every single family and to the shops also. Despite the considerable number of actions taken by the State Government and other private offices numerous territories have not been furnished with the dustbins and these areas have no entrance to the waste assortment centre points, because of which they dump their loss along the side of the road, empty land or into the jhoras making a horrendous situation in the district. Indeed, even way to entryway assortment of waste has been acclimated along the National Highway as well as has been extended in and around the encompassing spots of Gangtok like Sitchey, Development Area, Burtuketc on open solicitation. Still a few spots are lingering behind because of deficiency of trash vehicles and men power. The issue is additionally irritated by the troublesome territory which denotes the slope town. Gangtok comes up short on an urban arranging body. Accordingly, the metropolitan territories have grown arbitrarily, bringing about the improvement of zones where fundamental administrations like ambulances, trash assortment trucks, and fire engines can't reach to every one of the houses which has been worked in aimless way inside the tough territory. As indicated by the writing, this is run of the mill for creating countries. The individuals who live in these regions are compelled to toss their loss in the close by jhoras, since there is a deficiency of room for creating available assortment site. Climate also plays a crucial role for the collection of garbage from the houses which have been built on the hill tops. Rain and difficult terrain makes the back packers more difficult to reach out to people residing on the offside of the road making them forced to dump or throw their garbage in their local nalas and jhoras. These garbage back packers are even not provided by proper raincoats, rain boots and gloves to make them easy to work during the rainy season. When asked to the authority about the common necessities to the garbage back packers they answered me saying that they are provided but it seems they find it difficult to work with these gargets.

Transportation

On the everyday nuts and bolts at a fixed time trucks are utilized to ship the loss from the assortment places to the dumping yard. Gangtok alone has administration of around 7 trucks which chip away at everyday fundamentals for the assortment of waste in and around the town. All these seven trucks are structured as open waste vehicles. It is assumed that waste is gathered every day from over the civil territory and taken to the dumping yard. It was seen that the waste is shipped in open dump trucks and tends to tumble off at each knock or pothole out and about, as the street state of our street isn't in acceptable condition all over. We can likewise observe traffic brought about by these trash open trucks which makes horrendous to the individual who has the vehicle next to the trash vehicle because of it foul smell. Even the narrow lanes and clustered settlements inside the municipal areas make the garbage collectors difficult to reach the paces to collect the waste, sometimes making it impossible to collect the waste.

Dumping and Handling

On the day-by-day fundamentals at a fixed time trucks are utilized to ship the loss from the assortment destinations to the dumping yard. Gangtok alone has administration of around 7 trucks which deal with everyday essentials for the assortment of waste in and around the town. All these seven trucks are planned as open dump trucks. It is assumed that waste is gathered regularly from over the city zone and taken to the dumping yard. It was seen that the waste is moved in open dump trucks and tends to tumble off at each knock or pothole out and about, as the street state of our street isn't in acceptable condition all over the place. We can likewise observe traffic brought about by these trash open trucks which makes excruciating to the individual who has the vehicle other than the trash vehicle because of it foul smell. The Gangtok Municipal Corporation dump the collected garbage in anunloadingarea located 18km away from the centre ofthe town which is near Martan on NH 31A. It's an open dumping ground abutting the neighbourhood of Rani Khola. This is the minimal effort strategy on waste removal which just requiresa large open land to lay the dump squander. The practice has been disposed off by numerous countries worldwide as open dumps has an antagonistic impact on the earth causing soil and water contamination, drawing in numerous infections, dirtying condition with its foul smell, bringing forth flies and making homes for stray dogs. Due to poor management the local public as well as the passengers passing through the national highway along the dumping site must suffer bad smell from the spot. There is many sickness as well as illness spreading in the areas nearby. Reported many tuberculosis cases as well as cancer and breathing difficulties are spreading with rapid growth. As per the report by a local newspaper (Sikkim Chronicle dated 31st Jan 2020) the trailer machines for turning waste

materials into bio compose and organic manure installed is not working. Carelessly the garbage has been dumped without any proper treatment and without segregation.

Potential for Sustainable Solid Waste Management

The Union Government of India told the Municipal Solid Wastes (Management and Handling) Rules (2000) under Sections 3, 6 and 25 of the Environment (Protection) Act (1986), to oversee civil and metropolitan misuses/trash in an ecologically strong way [9]. The Union Ministry of Environment, Forests and Climate Change (MoEF&CC) lately informed the new Solid Waste Management Rules (SWM), 2016. "These will replace the Municipal Solid Wastes (Management and Handling) Rules, 2000, which have been in place for the past 16 years." "These rules are the sixth category of waste management rules brought out by the ministry, as it has earlier notified plastic, e-waste, biomedical, hazardous and construction and demolition waste management rules."

Development of Strong Waste Administration Framework in Gangtok

The PPP modelis appreciated in most quarters. The state is heading in the direction which focuses on the treatment, regulation and to monitoring the performance involved in the disposal of waste taken up by private sectors. Local communities and NGOs are promoted by state to collect the waste and transmit it to the authorized transfer station. Watson Committee and Golden Circle, namely the two NGOs are involved in the collection of household waste in the town. So far, the partnership looks productive, and the department is working up to encourage such ventures. The UD&HD department also aims to promote people's involvement under the Jawaharlal Nehru Urban Renewal Mission (JNURM)which may integrate the tenets of the segregation of waste and 3R's.The Gangtok Municipal Corporation has also made the regulation for the people that it may not collect the garbage from a household, if the waste is not segregated at the source. On the other hand, GMC garbage trucks has also fixed yellow colour bags at the back of 10-11 GMC trucks where used sanitary napkins and baby diapers are requested to be dumped. The sanitary pads and baby diapers are to be wrapped in paper so it can be identifying and managed accordingly. GMC has also pointed out that e-waste needs to be managed properly as e-waste contains hazardous elements. The government has a policy in place for e-waste but till date only e-waste from the government centres is coming to the e-waste collection centre while citizens are dumping waste mixing it with other waste items which is causing problem at the landfill site.

Summary, Conclusions and Recommendations

Among the numerous variables impacting strong waste administration, five key elements were recognized:

1) Basic leadership process.
2) Open impression of the waste issue.
3) Absence of straightforwardness and data sharing.
4) Connection between political security and administration, and
5) Self-sorted out grass roots level associations.

It was discovered that fundamentally, basic leadership is top-down and bureaucratic. There is a gap between leaders and the individuals as far as data change [11]. Most of the individuals are not educated about the choice attempted by the specialists. Individuals by and large don't know about the choices made and the district doesn't include open for conversation or conference. It brings about most of the executed plans flopping because of the absence of open help and interest. Long haul supportability of the strong waste administration framework additionally relies upon the degree of isolation of waste [12]. Isolation of waste ought to be three streams for example bio-degradable, recyclables and trash/waste; this will likewise help in finding suitable removal choices. Isolation of waste ought to be done at the source itself. Isolated waste can be gathered on a week-by-week premise from families and consistently from business foundations [13]. Emphasis ought to be set on the three R s – decrease, reuse, and reuse. This will help in making of less waste and in expanded material recuperation [14]. Decrease can be accomplished by beginning a store discount framework, for example it ought to be caused mandatory for specific kinds of waste to be dealt with by the organization creating them under expanded maker's duties. To guarantee that these specific squanders return

to the makers, an additional store (20-30% of the cost) could be charged when somebody buys these things, and this store ought to be recoverable on return of the things (state spread/foil/plastic containers and so forth) [15]. Bureaucrats are the foundation of any respectful association. If there should be an occurrence of unstable and constantly changing political circumstances, the administrators should assume a star dynamic job in guaranteeing that the tasks and plans and procedures are not influenced by the pervasive political circumstance [16]. Because of an adjustment in government at the city level, arrangements ought to be made to granddad the plans and procedures, began by the past board. Plans and procedures should not be permitted to be deserted halfway, which brings about loss of time and assets. Organization can assume a unique job in guaranteeing the smooth working of the district and this component ought to be remembered for the arranging procedure itself.

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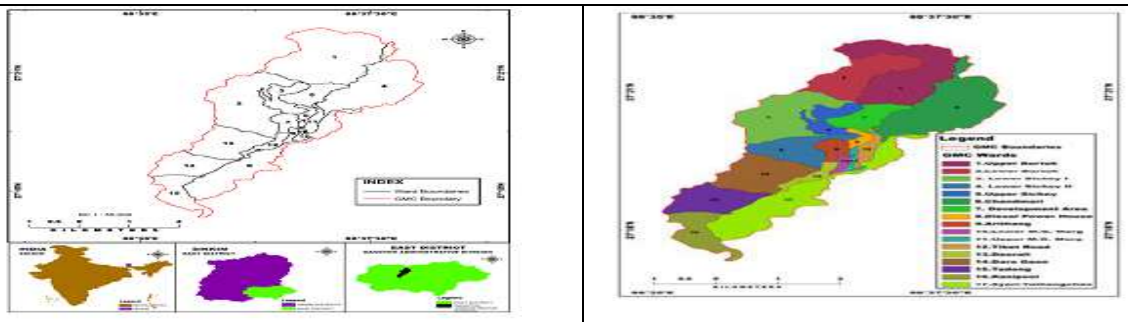


Fig. 1: Location map of Gangtok and its Municipal areas
Source: Map made by author

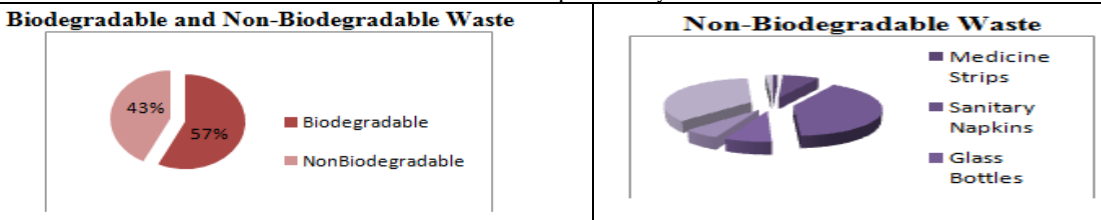
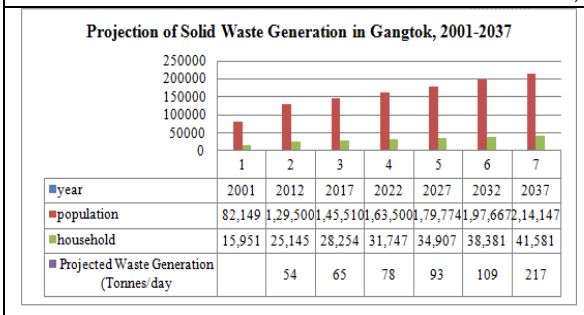


Fig. 2: Composition of Waste – Sikkim, 2011
Source: UDHD, Govt. of Sikkim, 2011



	1	2	3	4	5	6	7
year	2001	2012	2017	2022	2027	2032	2037
population	82,149	1,29,500	1,45,510	1,63,500	1,79,774	1,97,667	2,14,147
household	15,951	25,145	28,254	31,747	34,907	38,381	41,581
Projected Waste Generation (Tonnes/day		54	65	78	93	109	217

Fig. 3: Projection of Solid Waste Generation in Gangtok
Source: UDHD, Govt. of Sikkim, 2011

Fig 4: Images showing waste collection in and around Gangtok
Source: Author

Fig 5:Different types of dustbins used for waste collection in Gangtok
Source: Author

Fig 6: Open dumping yard where segregation of waste done manually
Source: Author

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RESEARCH ARTICLE

A Study of Ant Diversity (Hymenoptera: *Formicidae*) in two sites of Post Chromite Mining Ecosystem

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ABSTRACT

The present study deals with the diversity of ants in Post Chromite Mining Ecosystem. Two years of survey was conducted on the biodiversity of ant species in Post Chromite Mining ecosystem in Thagaduru village situated in Channarayapattana taluk, Hassan district of Karnataka, India. Ants were collected from two different habitats within varying disturbances level with the help of pitfall trap and hand collection methods from the afforested mined out sites and disturbed mined out sites in the Post Chromite Mining ecosystem. Total 22 species of ants were collected from afforested mined out area and 17 species were collected from disturbed mined out area. Afforested mined out sites contributes 5 subfamilies they are Formicinae with 5 species, Myrmicinae with 11 species, Ponerinae, Dolichoderinae, and Psuedomyrmicinae with 2 species each. While disturbed mined out area contributes 4 subfamilies they are Formicinae with 5 species, Myrmicinae with 9 species, Ponerinae with 2 species and Dolichoderinae with 1 species. Ant species *Meranoplus bicolor, Technomyrmex albipes, Tetraponera rufunigra* and *tetraponera alborans* were found to be absent in disturbed mined out habitats. The diversity and occurrence of ant species were assessed by Shanon- wiener index (H), Simpson index (D) and Berger Parker index (d).

Keywords: Ant, Diversity, Post Chromite Mining ecosystem, Afforestation, Shannon-wiener.

INTRODUCTION

Ants are ubiquitous insects prefer to live in almost all terrestrial habitats except in Antarctic zone. Habitats of ants have been altered by many natural events like fire and floods. The habitat alteration also happens by anthropogenic activities like mining, agriculture extension, real estate works and grazing by cattle's. Habitat disturbance has a significant influence on the existence of a species in its locality with a specific ecological condition. The habitat disturbance removes biomass [Townson and Hildrew 1994] and affects the existing community in many ways and ants in particular. Ants are highly sensitive to disturbances like loss of diversity, changes in plant and animal composition, interactions, tropic level changes and introduction of new intolerable conditions. Due to these sensitivity of ants to environmental changes, these insects have been considered to be the power tools of bio-indicator to monitor the changes in the ecological conditions [Andersen and Majer 2004]. Ants play a central role in understanding the effects of habitats loss and fragmentation on community and ecosystem because of their diversity, abundance and functional roles in ecosystem. Mining is considered to be the most extreme form of habitat disturbance and transitory mode leading to habitat loss. Ants have been widely used as indicators for mine rehabilitation work. This indication helps one to compare range of rehabilitation sites varying in age with disturbed mined out site comparison. The rehabilitation many times is associated with increasing the heterogeneity and habitat in relation to species richness and vegetation [Anderson *et al*., 2003, Majer *et al.,* 1984]. In this regard monocultures of plant found to be less supporting than mixed vegetation for the rehabilitation process. Even after rehabilitation efforts complete recreation of the lost habitat has become impossibility. Thereby mining leaves biggest impression of damage of habitat and destruction of ant's species richness [Hoffman *et al.,* 2000]. In Indian contexts earlier works on ants are majorly on distribution [Bharati *et al.,* 2016], ecology (Varghese *et al., 2003*) existence of ants in agro ecosystem and agriculture fields predation on other organisms, habitat persistence [Mahalakshmi and Channaveerappa 2016) and behaviour. These studies have not examined the effect of mining and grazing on the existence of ants but for disturbance by agriculture in this regard this work on distribution of ants in afforested mined out and disturbed mined out ecosystems carries a significance to establish the effect of disturbance on survival of ants and which has to be considered as indicators of progressive survival and the same could be utilized to rebuilt the damaged ecosystem.

MATERIAL AND METHODS

Sample collection location

The Post Chromite Mining ecosystem is situated in Thagaduru village, Channarayapattana Taluk, Hassan district, Karnataka, India. Its coordinates ranges between Longitude- 130 01′ 50″ E to 130 04′30″ E and Latitude -760 26′ 40″ N to 760 27′ 30″ N. Mine Lease Area - 614.99 ha. Type of the Area - Patta Land, Government land. Mining area was divided into two different habitats, they were afforested mined out sites and disturbed mined out sites. 10 different plots were selected in both afforested mined out sites and disturbed mined out sites in the post chromite mining ecosystem for the ant collection.

Afforested mined out area

10 different plots were selected for ant's collection. These spots are least disturbances area of Post Chromite Mining ecosystem. Human activities are nearly nil from this area. This region is having large number of trees, shrubs, and grasses. The vegetation mainly containing *Ecalyptus gradis and Acacia auriculiformis trees were* more dominant tree species in these sites. This vegetation also includes trees like *Azardicta indica, Psuedososa japonica, Ficus religiosa, Pongemia pinnata, Vachellia nilatica, Santalum album, Lantana camera, Agave tequilana, Vachelia tartilis, Acacia lenticularis, Vachelia reficienus, Butea suparba, Cyclobalanopsis champi, Mangifera indica, Antocarpus heterophyllus, Tamarindus indica* were in less number. Due to these trees there are good canopy covers and floor has high litter content unlike that of disturbed mined out site.

Disturbed mined out area

Similarly 10 plots were selected for collection of ants as a representative. These spots are heavily disturbed because of anthropogenic activities like mining, grazing, digging of mining pits, cracking of rock explosures, and much noise disturbance from the vehicles used for mining purposes and transportation. Deforestation is also the reason for the disturbances. The habitat from this region is adversely affected by human activities. This habitat predominantly includes grasses, shrubs, and only few tree species which includes *Acacia cornizera, Acacia holosericeae, Dodonea viscose, Acacia angustissinus, Mimosa glarca, Lantana camera, Acacia auriculiformis, Senegalia senegal, Cyclobalanopsis championii.*

Sampling methods

The ant species were collected by pitfall trap and hand collection methods from January 2020- December 2021. Pitfall traps containing sugar syrup in plastic beverage bottles with an opening of 9cm in diameter are buried at ground level. One pitfall trap was placed in each 10m randomly chosen 10m×10m quadrates of one hectare plot in between 7.30 am to 9.0 am. Traps were collected after 48 hours (Gadagkar *et al.,* 1993). In addition to that hand collection of ants from each sampling plot was carried out for 30-40 minutes before the traps were collected in all sampling days to collect representative's individuals of all species seen in the quadrate after laying the baits (Ramrao *et al.,* 2014). For removal of sampling error we used 2 different methods to collect the maximum number of ant species from study area. The collected ant species were sorted, washed and preserved in 70% Alcohol in separate plastic vials and brought to the laboratory for identification. Ants were photographed by using Zeiss Stereo Discovery V.20 Stereo Microscope. The collected ants were identified up to the genus level with the help of Senior Associate Scientist Thresiamma Varghese, Center for Ecology, IISc, Bangalore. Identified ants were preserved by 2 methods.

1. Wet preservation method: - Identified species were preserved in separate vials individually Containing 70% alcohol.
2. Dry preservation method: - Identified ant species were preserved by pinning method.

Species diversity was calculated by using Shannon-Wiener diversity index (H), Simpson's (D) diversity index and Berger-Parker diversity index (d). Analysis was done by using Paleontological Statistics Software Tool (PAST).

RESULT AND DISCUSSION

In the present study area a total of 22 ant species (from 1204 ants) with 15 genera from 5 subfamily were reported as evident from Table 1. A total of 22 and 17 ant species were collected from Afforested mined out sites and disturbed mined out sites respectively as evident from table 1 and table 2. Out of 22 ant species almost 17 species (77%) were common to both study sites, while another 5 species (23%) were found exclusive to the afforested mined out ecosystem. From a total of 22 ant species *Solenopsis invicta, Technomyrmex albipes, Meranoplus bicolor, Tetraponera rufunigra, and Tetraponera alborans* were not reported from disturbed mined out sites. The number of ants collected from afforested mined out sites (704) were more as compared to the disturbed mined out sites (500) as shown in Table-3. In afforested mined out sites subfamily Myrmicinae (11 species) was more diverse and most abundant sub family with 7 genus and 11 species then followed Formicinae (3 genera and 5 species), Ponerinae (2 genera and 2 species), Dolichoderinae (2 genera and 2 species), and Pseudomyrmecinae (1 genera and 2 species) each as shown in Figure 1. Percentage of ants from different sub families at Afforested mined out ecosystem Myrmicinae (50%) followed by Formicinae (23%) and other three subfamilies Ponerinae (9%), Dolichoderinae (9%) and Psuedomyrmicinae (9%) shows the same percentage with two genera and two species each as evident from Figure 3. Whereas in disturbed Mined out sites subfamily Myrmicinae (6 genera and 9 species) was more diverse then followed Formicinae (3genera and 5 species), Ponerinae (2 genera and 2 Species) and, Dolichoderinae (a genera and a Species) least diverse comprising only with one species as evident from Table 2. Species richness and abundance of ants from disturbed mined out sites was subfamily Myrmicinae showed more species abundance with 295 species from 6 genera than other 3 subfamilies in Post Chromite Mining Ecosystem as evident from Table 3. Shannon-Wiener diversity index (H) for afforested mined out sites (2.92) was slightly higher than that of disturbed mined out sites

(2.65). Similarly, Simpson's index (D) for afforested mined out sites is 0.940 while, for disturbed mining site is 0.920. Berger-Parker (d) diversity index for afforested mined out sites (0.089) was slightly higher than that of disturbed mined out sites (0.126). Species composition, abundance and evenness from each of the habitats were considerably different as evident from table 4. Four sub families with the number of genus and species of ants collected from disturbed mined out ecosystem as shown in the Figure 2. Percentage of ants from different sub families at Disturbed mined out ecosystem Myrmicinae (53%) followed by Formicinae (29%) followed by Ponerinae (12%) and subfamily Dolichoderinae (6%) with only one genera and a species. This shows very less diversity of ants from disturbed mining ecosystem as evident from Figure 4. The most abundant ant species from afforested mined out sites are *Pheidole watsoni, Pheidole xerophila, Tapinoma melanocephalum, Camponotus compressus, Camponotus irritasns, Camponotus sericeus, Paratrechina longicornis, Solenopsis geminate,* and *Technomyrmex albipes.* These species having nest on the grounds, under rocks, under the leaf litter and inside soil. Due to the less anthropogenic activities, no grazing, no noise pollution and no mining activities in these afforested mined out sites provides a good leaf litter, shady nests, humidity, less temperature and all the essentials which ants needs for their existence. Presence of large trees provide habitats in their roots, stem, bark and branches of the tree for arboreal ants like *Crematogaster cerasi, Crematogaster lineolata, Tetraponera rufunigra, tetraponera alborans* and *Tapinoma melanocephalum* in this area.

In disturbed mined out ecosystem ground nest ants *Cerebera diversa, Pheidole xerophila, Pheidole watsoni, Camponotus compressus, Paratrechina longicornis, Solenopsis geminate. Pheidole* species, *Camponotus compressuss,* were dominant, these species found all over within the disturbed sites with bigger range. All these species having their nest on the ground especially on sandy and stone variety of soil. These species will survive all unfavorable climates. Overall relative abundance of Myrmicinae from disturbed mined out sites was a more as a result of that they will have high potential to adapt to variable environmental conditions and that they square measure found in numerous form of habitats worldwide. Relative abundance of *Cerebera diversa, Pheidole xerophila, Pheidole watsoni, Camponotus compressus, Paratrechina longicornis, Solenopsis geminate w*ere high within the disturbed mined out sites as shown in Table 1. This is because of presence of small habitats that square measure ideal for higher than above mentioned ant species. *Tapinoma melanocephalum* is from Dominant Dolichodrinae (DD) purposeful cluster and that they like hot and open habitats. They are exceptionally active, aggressive and motion a robust competitive influence on different ants (Suriyapong, Y., 2003). *Solenopsis geminate* are categorized as Cryptic species functional group by Andersen (2000) and relative abundance is increased in vulnerable to the establishment of introduced ant species (TschinkeI1988; Suarez *et al.* 1998). From species diversity indices it can be concluded that the ant diversity is varied in both type of habitats. This is due to ant species richness and abundance may change with the canopy cover, habitat complexity and level of disturbance.

CONCLUSION

From over all results that species richness, diversity and abundance were higher in afforested mined out sites as compared to disturbed mined out site. This is often because of surrounding destruction and increase in disturbance by varied anthropogenic activities in disturbed mining space. Connected studies on ants, birds and butterflies have shown that species richness and variety decreases with increase in disturbance (Andersen 1995; Blair 1996; Ingalhallikar et al. 2000-2001; Kunte 2000-2001; Pachpor & Ghodke 2000-2001). Several studies from completely different regions of world have shown that surrounding degradation, disturbance and fragmentation have a negative impact on hymenopteran diversity and abundance wherever afforested mined out sites has higher species richness than those in disturbed mined out sites (Greenslade and Greenslade, 1977; Olson, 1991; Suarez et al., 1998; Vasconcelos, 1999; Watt et al., 2002). Surrounding variables like cover cowl associated litter content in the soil will offer an acceptable surrounding for ants. This is often thanks to surrounding complexness and no uniformity was high with in the conversionsite as compared to disturbed mined out sites. Surrounding complexness provides activity, nesting and search grounds to the numerous hymenopterans species, however disturbed mined out sites doesn't. Habitat complexity provides hiding, nesting and foraging grounds to the many ant species, but disturbed mined out sites does not. Ants are often employed as biological indictors because they instantly indicate on any

alteration within the close surroundings. Based on sure hymenopteran species in surroundings one can able to access role of pollution, so in this connection elaborate studies of disturbed habitats are desperately required.

RESULTS AND DISCUSSION

Table 1:- Species and Sub family of ants collected from Afforested mined out sites. Table 2:- Species and Sub family of ants collected from disturbed mined out sites. Table 3. Species richness and abundance of ants from Afforested mined out sites and disturbed mined out sites from Thagaduru Post Chromite Mining Ecosystem, Hassan, Karnataka, India. Note: Indicated Figures in bracket indicates abundance of Ants. Table 4. Shannon's-Wiener diversity index(H), Simpson's diversity index (D), Berger –Parker index(d) and evenness of ants from Afforested mined out sites and Disturbed mined out sites from Thagaduru Post Chromite Mining Ecosystem, Hassan, Karnataka. India. Fig. 1. Sub families with the number of genus and species of ants collected from Afforested mined out ecosystem. Fig. 2. Sub families with the number of genus and species of ants collected from disturbed mined out ecosystem. Fig. 3. Percentage of ants from different sub families at afforested mined out ecosystem. Fig. 4. Percentage of ants from different sub families at Disturbed mined out ecosystem.

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Table 1:- Species and Sub family of ants collected from Afforested mined out sites.

Sl.No.	Species name	Subfamily
1	*Camponotus compressuss*	Formicinae
2	*Camponotus irritans*	
3	*Camponotus sericeus*	
4	*Polyrhachis armata*	
5	*Paratrechina longiceps*	
6	*Monomorium pharaonis*	Myrmicinae
7	*Monomorium minimum*	
8	*Tetramorium caespitum*	
9	*Crematogaster cerasi*	
10	*Crematogaster lineolata*	
11	*Solenopsis geminata*	
12	*Solenopsis invicta*	
13	*Pheidole watsoni*	
14	*Pheidole xerophila*	
15	*Cerabera divarsa*	
16	*Meranoplus bicolor*	
17	*Diacamma ceylonense*	Ponerinae
18	*Leptogynus processionalis*	
19	*Tapinoma melanocephalum*	Dolichoderinae
20	*Technomermex albipes*	
21	*Tetraponera rufunigra*	Psuedomyrmicinae
22	*Tetraponera alborans*	

Table 2:- Species and Sub family of ants collected from disturbed mined out sites.

Sl.No.	Species name	Subfamily
1	*Camponotus compressuss*	Formicinae
2	*Camponotus irritans*	
3	*Camponotus sericeus*	
4	*Polyrhachis armata*	
5	*Paratrechina longiceps*	

6	*Monomorium pharaonis*	Myrmicinae
7	*Monomorium minimum*	
8	*Tetramorium caespitum*	
9	*Crematogaster cerasi*	
10	*Crematogaster lineolata*	
11	*Solenopsis geminata*	
12	*Pheidole watsoni*	
13	*Pheidole xerophila*	
14	*Cerabera divarsa*	
15	*Diacamma ceylonense*	Ponerinae
16	*Leptogynus processionalis*	
17	*Tapinoma melanocephalum*	Dolichoderinae

Table 3. Species richness and abundance of ants from Afforested mined out sites and disturbed mined out sites from Thagaduru Post Chromite Mining Ecosystem, Hassan, Karnataka, India.

Note: Indicated Figures in bracket indicates abundance of Ants

	Study sites	
Subfamily	**Afforested mined out sites**	**Disturbed mined out sites**
Formicinae	5 (226)	5 (147)
Myrmicinae	11 (295)	9 (229)
Ponerinae	2 (56)	2 (28)
Dolichoderinae	2 (90)	1 (26)
Pseudomyrmecinae	2 (40)	-
Species richness	21 (704)	17 (500)

Table 4. Shannon's-Wiener diversity index(H), Simpson's diversity index (D), Berger –Parker index(d) and evenness of ants from Afforested mined out sites and Disturbed mined out sites from Thagaduru Post Chromite Mining Ecosystem, Hassan, Karnataka. India.

	Study sites	
	Afforested mined out sites	**Disturbed mined out sites**
Shannon-Wiener diversity(H)	2.92	2.65
Simpson's index (D)	0.940	0.920
Berger-Parker index(d)	0.089	0.126
Eveness (eH)	0.847	0.8335

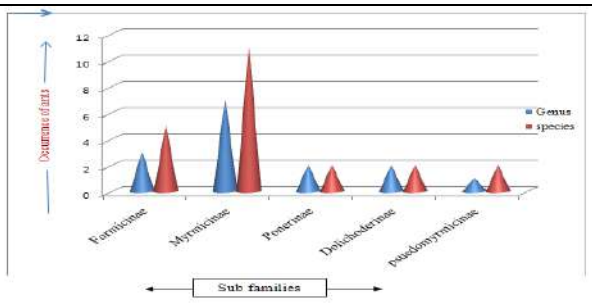

Fig. 1. Sub families with the number of genus and species of ants collected from Afforested mined out ecosystem.

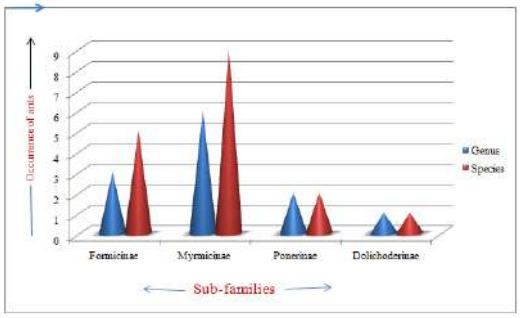

Fig. 2. Sub families with the number of genus and species of ants collected from disturbed mined out ecosystem

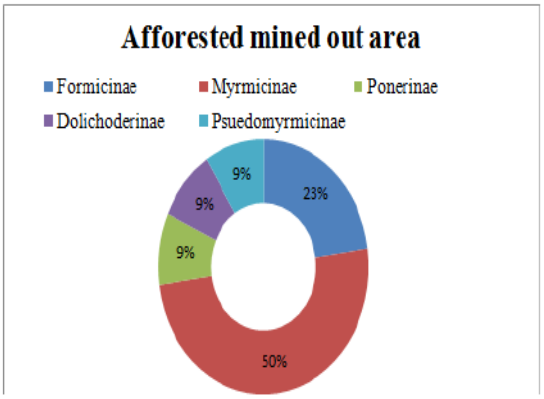

Fig. 3. Percentage of ants from different sub families at afforested mined out ecosystem.

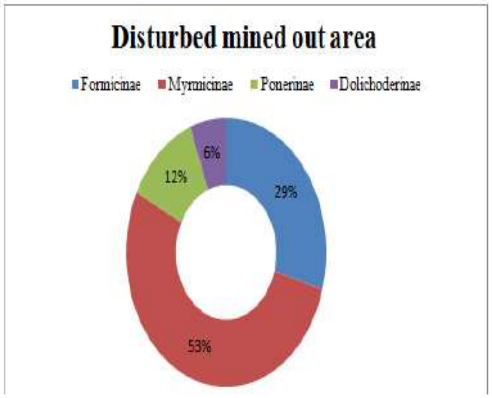

Fig. 4. Percentage of ants from different sub families at Disturbed mined out ecosystem.

RESEARCH ARTICLE

Control Chart using Zubair Exponential Distribution

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ABSTRACT

Quality control methods have been developed by introducing a new family of distribution called Zubair Exponential. Under the assumption that the variable does not follow normal distribution. Control limits have been obtained and illustrated with a real life data.

Keywords: Statistical Quality Control; Control Limit; Zubair Exponential Distribution.

INTRODUCTION

Quality has been proven to be one of the most important aspects of a company's success. From manufacturing to service industries, the number of sales and thus the profit of the company is determined by the quality of the product or inspections provided. However, the quality of one product/service cannot be compared to that of another. Nonetheless, it is the design and development department's responsibility to ensure that the differences between specifications and actual values are as small as possible. As a result, statistical quality control techniques are used to improve the product/service quality. In general, quality refers to a product's suitability for end-user use. In order to achieve a high level of quality, special attention is paid to and emphasis is placed on the manufacturing of a product with faults that are kept to a minimum. This means that a product must perform as intended under the given conditions for a specified period of time, to the best of one's ability.

The quality of a product is carefully monitored in order to achieve this. Where the product's performance is compared to industry standards. Quality control techniques were reinforced and developed by the American Society for Quality. The science of statistics is defined as a quality control technique that employs statistical tools to control,

enhance, and resolve any quality problems. Its first applications were seen in the chemical industry, but it later spread to other industries. Statistical quality control techniques are one of the quality control methods that takes variations into account. It entails determining abnormal variations during process monitoring, identifying factors that cause these variations, and determining how to reduce them. It is difficult to maintain quality because it is not a constant factor but rather one that changes depending on circumstances. As a result, quality and variability are inversely proportional. It is clear from this statement that statistical methods are the outcome of quality improvement. By assuming that the variable follows a Normal distribution, one can reduce variation by removing the special cause of variation or removing the effects that cause variation. In this paper, quality control methods for non-normal distributions have been developed using the Zubair Exponential of distribution for various situations. The following is a breakdown of the paper's structure. Section 2 examines the relevant literature. Section 3 provides a description of the Zubair Exponential distribution as well as measures. Section 4 provides the control limits. The process of identifying out of control is illustrated with real-life data in Section 5. Section 6 contains a summary.

Literature Review

*T*he SPC was created to deal with variation in production, where two products manufactured cannot be exact replicas of each other. When there are small deviations that are difficult to identify due to random causes, the process is said to be "under control," and when the deviations are noticeable and the cause is active, the process is said to be "out of control." The products are sent to the system and await inspection during process monitoring. Process control also protects the product because it allows you to keep batches within acceptable quality limits. Prof. Walter Shewhart developed the Shewhart control charts, which have been widely used since the 1920s. Several researchers have since developed various control charts. They produce control charts based on the assumption that the process is being monitored, and they produce a quality property that is closer to a symmetric normal distribution if the system only has natural sources of variation. The central limit theorem can be used to estimate the distribution of a normal distribution if the measure and monitored samples are large enough. However, in many industrial situations, this cannot be guaranteed, and the process's output does not always have to be distributed normally and skewed. Control charts based on normality assumptions are used in cases where the distribution is asymmetric, but this leads to incorrect conclusions about the process's stability. Such erroneous assumptions would result in faulty products, causing manufacturers and customers to lose money to competitors.

As a result, other types of distribution are still required in the context of quality control methods. By including distribution that provides a good quality model managed by the industry, statistical information and warnings about computing devices can easily solve this problem. Eduardo and Joel (2013) proposed using the transformation of the variable as proposed by Nelson(1994) that converted exponentially distributed data into estimated normal data. Karagoz and Hamurkaroglu (2012) used some non-normal distributions like lognormal, and Eduardo and Joel (2013) proposed using the transformation of the variable as proposed by Nelson(1994) that converted exponentially distributed data into estimated normal data. SPC using Transmuted Generalized Uniform distribution was introduced by Amin and Venkatesan (2019) to determine if the process is under control. Although manufacturing products are typically produced with a defined variance and nominal value, unexpected events do occur, which may result in changes in the manufacturing process. Different types of distribution are introduced and used to develop a control chart to study the efficiency of a production process when quality characteristics do not follow a normal distribution in this paper.

The distribution's description

Definition: A random variable X has a Zubair Exponential distribution if the density function is given by

$$f(x,\alpha,\lambda)=\frac{2\alpha\lambda e^{-\lambda x}\left(1-e^{-\lambda x}\right)e^{\left\{\alpha\left(1-e^{-\lambda x}\right)^2\right\}}}{e^{\alpha}-1}\quad \lambda>0, x>0, \alpha>0 \qquad (1)$$

Where α and λ are the shape and scale parameters of the distribution respectively and its moment generating function can be obtained by

$$M_X(t)=\int_0^{\infty} e^{tx} f(x)dx$$

$$\int_0^{\infty} \frac{e^{tx}\,2\alpha\lambda e^{-\lambda x}\left(1-e^{-\lambda x}\right)e^{\left\{\alpha\left(1-e^{-\lambda x}\right)^2\right\}}}{e^{\alpha}-1}dx$$

=

One gets after simplifications

$$M_X(t)=\frac{2\alpha e^{t+\alpha-\lambda}}{e^{\alpha}-1} \tag{2}$$

In order to use this distribution, one need study the mean and variance of the distribution. The mean variance are obtained are respectively are given as follows,

$$E(X)=\frac{2\alpha}{e^{\alpha}-1}\,\mathrm{e}^{\alpha-\lambda}\left(\alpha-\lambda+1\right) \tag{3}$$

$$V(X)=\frac{\alpha e^{\alpha-\lambda}}{e^{\alpha}-1}\left(2(1+\alpha-\lambda)\left(1+\frac{2\alpha e^{\alpha-\lambda}}{e^{\alpha}-1}(\alpha-\lambda+1)\right)+(\alpha-\lambda)^2\right) \tag{4}$$

Control limits

Three sigma UCL and LCL are derived as the basis of the standard format as specified in Montgomery (2012) and one can get the control limits for Zubair Exponential distribution using (3) and (4) and are given by

$$UCL=\frac{2\alpha}{e^{\alpha}-1}e^{\alpha-\lambda}(\alpha-\lambda+1)+3\sqrt{\frac{\alpha e^{\alpha-\lambda}}{e^{\alpha}-1}(2(1+\alpha-\lambda))\left(1+\frac{2\alpha e^{\alpha-\lambda}}{e^{\alpha}-1}(\alpha-\lambda+1)\right)}+(\alpha-\lambda)$$

$$CL=\frac{2\alpha}{e^{\alpha}-1}e^{\alpha-\lambda}(\alpha-\lambda+1)$$

$$LCL=\frac{2\alpha}{e^{\alpha}-1}e^{\alpha-\lambda}(\alpha-\lambda+1)-3\sqrt{\frac{\alpha e^{\alpha-\lambda}}{e^{\alpha}-1}(2(1+\alpha-\lambda))\left(1+\frac{2\alpha e^{\alpha-\lambda}}{e^{\alpha}-1}(\alpha-\lambda+1)\right)}+(\alpha-\lambda)$$

Numerical Illustration

In order to illustrate practicability of the proposed method, an example regarding the construction of control limits is considered. The control limits for Zubair Exponential distribution are obtained by using simulated data set for different values of the parameters. When the sample size 5000. Therefore, UCL and LCL can be obtained are given in Table1.As one can observe from the in Table 1, for the parameter α, the control limits increase whenever the scale parameter λ increase. In addition, for the increasing of parameter α with fixed scale parameter λ,the control limits also increase. The below fig.1. Show the Zubair Exponential distribution control limit $\lambda=5,\alpha=3$

CONCLUSIONS

The control chart in this paper is based on the Zubair Exponential distribution. When a variable is assumed to have a non-normal distribution. The control limits for the Zubair Exponential distribution with various parameter values and are given. The table is designed to aid in the selection of parameters based on the type of data that the manufacturing engineer considers important. The control chart is drawn by considering the following parameter and observing that the 21st observation is out of control because the value of parameter is greater. As a result, it's best to use process data that's less than the value of parameter. When the distribution is not normal, the proposed model perfectly detects the out of control point at the 21st observation.

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Table 1: Control limits Using Zubair Exponential distribution

α	λ	UCL	CL	LCL
3	1	20.08	6.96	-2.14
	2	9.16	1.71	-3.75
	3	11.42	0.31	-10.79
5	1	79.60	18.51	-34.56
	2	26.26	6.45	-9.36
	3	9.33	1.50	-2.33
	4	3.49	0.36	-0.76
	5	0.26	0.06	-0.12
7	1	151.79	36.08	-67.72
	2	8.38	11.38	-8.82
	3	20.51	3.49	-5.53
	4	8.38	1.03	-0.32
	5	4.14	0.28	-0.42
	6	1.94	0.07	-0.20
	7	0.43	0.01	-0.41
9	1	248.17	59.60	-112.97
	2	87.49	19.49	-32.51
	3	32.58	6.27	-8.04
	4	14.15	1.98	-0.19

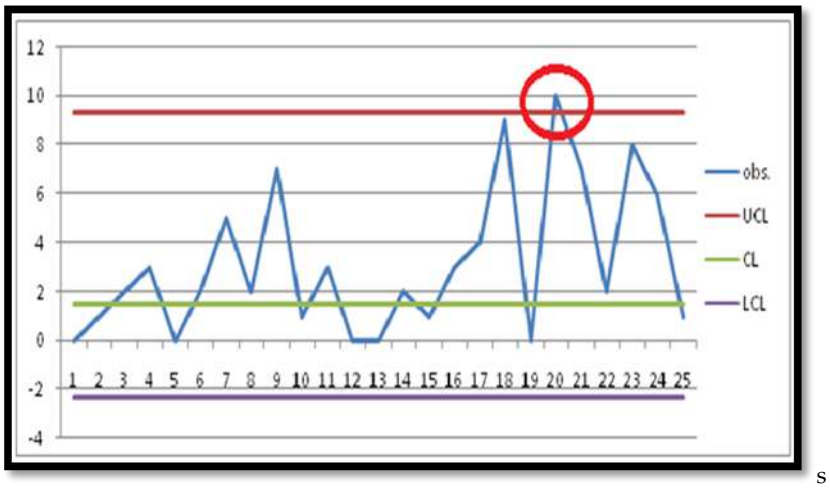

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Fig.1. Show the Zubair Exponential distribution control limit $\lambda = 5, \alpha = 3$

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RESEARCH ARTICLE

Influence of Integrated Nutrients on Flowering, Flower Yield and Quality of Gerbera (*Gerbera jamesonii* Bolux.)

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ABSTRACT

An experiment was conducted to study the "Effect of integrated nutrients on flowering, flower yield and quality of Gerbera (*Gerbera jamesonii Bolux ex Hooker f.) Cv.* pink elegance" under naturally ventilated poly house condition was carried out at Muthalli, Hosur, Dharmapuri district, during the period 2020-2021. The experiment comprised of various treatments with different doses of recommended fertilizer (50, 75 and 100 percent), basal application of organic fertilizers (Farmyard manure and Coirpith compost each @ 5 kg m^{-2} and Vermicompost @ 2.5 kg m^{-2}) along with *Azospirillum and Phosphobacter* each @ 20 g m^{-2} and foliar spray of biostimulants (Humic acid, Panchagavya, Vermiwash, Sea weed extract) each @ 2 percent concentration. Among the treatments, application of 75 percent recommended dose of fertilizer + Farmyard manure and Coirpith compost each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + foliar spray of Humic acid @ 2 percent was significantly increased flowering, yield and quality parameters *viz.*, days taken for emergence of flower bud (23.81), days to full bloom of flowers (11.94), days taken for fifty percent of flowering (71.58), diameter and length of ray florets (10.15 cm and 3.96 cm), length and diameter of flower stalk (50.10 cm and 0.491 cm), number of flowers $plant^{-1}$ (29.44), number of flowers $plot^{-1}$ (235.52), number of flowers ha^{-1} (235520) and vase life (15.97 days) are observed in the T_7 with the application of 75 percent recommended dose of fertilizer along with farm yard manure and coirpith compost each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + foliar spray of Humic acid @ 2 percent. It was observed that the least values were obtained under control (Treatment T_1). In general, the treatment T_7 with the application of recommended dose of fertilizer 75 percent RDF + FYM and CP each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + foliar spray of Humic acid @ 2

percent could adjudged as the best treatment in performance of Gerbera (*Gerbera jamesonii Bolux ex Hooker f.) cv.* pink elegance in protected polyhouse condition than compared to other treatments.

Keywords: Gerbera, Organic fertilizers, Humic acid

INTRODUCTION

Flowers play a cardinal role in human behavior and bring tranquility and peace of mind to the people. With changing life style and increased urban affluence floriculture has assumed a definite commercial status more precisely modern cut flower gains additional attention. Currently, about 30 per cent of flower production is exported from India to other countries and the remainder sold on the domestic market. In India the major cut flowers are exported to European countries, Arabian countries, USA and other Asian countries. Gerbera (*Gerbera jamesonii Bolus ex.Hooker f.*) was discovered by pre-Linnaean botanist, Gronovious but it is named after German naturalist, "Traugott Gerber" who travelled Russia in 1743. Gerbera belongs to the family Asteraceae and which it is native to tropical Asia and Africa. It is an internationally important cut flower used worldwide grown for its showy and long lasting daisy like flowers, exquisite shape, size, and bewitching color. (Singh *et al.* 2017). It is a leading flower and ranks among the top ten cut flowers of the world with wider applicability in the flower industry as cut flower and potted plant. The cut flowers have a long vase life, which fetches premium market prices. The flowers are hardy and withstand transportation stress to an extent. Flower longevity depends on the stage of harvest. Nutrient management is considered to be an important technology for the productivity of crop plants. Among the plant nutrients, nitrogen, phosphorous and potassium are the most important macro nutrient elements that decide the growth and yield of crops. When farm yard manure is incorporated in to soil they help to improve soil fertility status and reduces the cost of inorganic amendments (Jarvan *et al.* 2017). Another essential component of good farming is the use of biofertilizers along with both inorganic and organic fertilizers. They are capable of supplying nutrients from soil to plant system by their biological activity and are also used for maintenance of long term soil fertility and fostering soil biological activity . Biostimulant additive to fertilizers and support the uptake of nutrients, promote plant growth, and increase tolerance to abiotic stress. It provides macro nutrients, essential micro nutrients, many vitamins, required amino acids, growth promoting substances and beneficial microorganisms for well growth of plants (Calvo *et al*.,2014). Humic acid, Panchagavya , Seaweeds, Vermiwash are one of the important bio-resources which are nowadays termed as fantastically promising plant growth regulators. These contains all major and minor nutrients, trace elements, vitamins, auxins and other bioactive substances. These bio regulators have revealed the presence of a wide variety of plant growth promoting substances such as auxins, cytokinins and betaines. Moreover, the integrated plant nutrient supply system holds a great promise in meeting up the growing nutrient demands of intensive horticulture and maintains the crop productivity at a fairly high level. Keeping in the view of the above facts and the paucity of research on these aspects the present study on "Effect of integrated nutrients on growth and yield of Gerbera (*Gerbera jamesonii Bolus ex Hooker f.) cv*-pink elegance" was conducted with following objectives to find out the best treatment of integrated nutrients and to study the interaction effect of organic fertilizers, inorganic fertilizers, bio-fertilizers and biostimulants on flowering, yield and quality of Gerbera.

MATERIALS AND METHODS

The present Study entitled "Influence of integrated nutrients on growth, yield and flower quality of Gerbera (*Gerbera jamesonii) cv.*pink elegance" under poly- house condition was carried out in a farmer's field at Muthalli , Hosur, Dharmapuri district, during the period 2020-2021. The experiment was laid out in Randomized Block Design (RBD) with 14 treatments and replicated three times. The polyhouse used for the experiment was a commercial polyhouse with the natural ventilation. For the experiment as per the treatment schedule, the plants were treated with recommended fertilizer dose (RDF) for 50, 75, 100 percent .The organic input like farmyard manure, vermicompost

and coirpith compost and biofertilizers (*Azospirillum* and phosphobacter each @ 20 g m^{-2}) has been mixed along with garden soil and sand then as per treatment schedule it is treated with foliar application of biostimulants (Humic acid, Panchagavya, Sea Weed Extract, Vermiwash) each @ 2 percent were applied to the experimental field. The observations are recorded on the selected five plants for a treatment in each replication and the mean data is statistically analyzed. The plots were kept free from weeds by periodic hand weeding. Pests and diseases were controlled periodically during the entire crop period. The data were subjected to statistical analysis as suggested by Panse and Sukhatme (1985). Data of three replications were tabulated and recorded. The treatment details are shown in the Table 1

RESULTS AND DISCUSSION

The research study of Gerbera on the use of organic and inorganic nutrients significantly influenced the flowering, yield and quality parameters. The data and the result on the "Effect of integrated nutrients on flowering, flower yield and quality parameters of Gerbera (*Gerbera jamesonii Bolux ex Hooker f.) Cv.* pink elegance" under naturally ventilated poly house condition and their biometric observation for flowering parameters *viz*., days taken for emergence of flower bud, days to full bloom of flowers, days taken for fifty percent of flowering, diameter and length of ray florets and length and diameter of flower stalk. The observation for flower yield and quality parameters *viz*., number of flowers $plant^{-1}$, number of flowers $plot^{-1}$, number of flowers ha^{-1} and vase life (days) were recorded and presented in Table 1 and 2.

FLOWERING PARAMETERS

Flowering parameters are those that have direct impact on yield and marketability of cut flowers. Flowering parameters includes days taken for emergence of flower bud, days taken for full bloom of flowers and days taken for 50 percent flower opening. The minimum days taken for emergence of flower bud were observed in the best treatment T_7 (23.87 days) with the application of 75 percent of recommended dose of fertilizer + farmyard manure and coirpith compost each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter @ 20 g m^{-2} + 2 percent Humic acid which was followed by T_9 at 23.98 days. Reducing the levels of chemical fertilizer and optimizing the dose of different organic nutrients can improve yield and quality in ornamental crops without adversely affecting the edaphic and environmental features. The early flowering is due to appropriate application of inorganic fertilizers in addition to farmyard manure and coirpith compost each @ 5 kg m^{-2} and biofertilizers as basal application to the garden soil might enhance early transformation of nutrients by increasing the flowering parameters. Balanced nutrition has considerable importance in improving the yield and reducing the duration of flowering. The earliness of flowering may also be attributed to the presence of biofertilizers especially inoculation with *Azospirillum* and Phosphobacter which resulted in easy uptake of nutrients and simultaneous transport of growth promoting substances like cytokinin to the axillary buds resulting in faster flower opening. Biostimulants offer a potentially novel approach for the regulation and/or modification of physiological processes in plants to stimulate growth, to mitigate stress-induced limitations, and to increase yield. The higher production of auxin and growth substances by humic acid at early phase of growth would have contributed to early flowering and reduction in the duration of flowering. Similar results are observed Sujatha Nair *et al*., (2020) in Dendrobium orchid, Bisnupada *et al*.,(2020) in Gerbera.

The various research workers have reported that the application of foliar spray of humic acid plant growth regulators along with appropriate quantity of organic and inorganic nutrients helps to produce the good quality of flower crops Minimum days of complete flower opening occur at 11.94 were recorded in Treatment T_7 (75 percent recommended dose of fertilizer farm yard manure and coirpith compost each @ 5 kg m^{-2} + 20 g m^{-2} of *Azospirillum* and phosphobacter + foliar spray of 2 percent Humic acid). Treatment T_9 with 12.07 days which was the next least number of days. The minimum number of days (71.58) taken for 50 percent flowering was observed in T_7 followed by treatment combination T_9 recorded with 72.43 days. .Minimum days taken for full bloom and days to 50 percent flowering ascribed due to easy uptake of nutrients and simultaneous growth promoting substances like cytokinins to the axillary buds resulting in breakage of apical dominance. Ultimately, they resulted in better sink for faster mobilization of photosynthates and early transformation of plant parts from vegetative to reproductive phase. These

ways the early days taken for full bloom of flowers is logically due the basal application of organic, inorganic nutrients and biofertilizers. At the same time these types of nutrients improve the soil physical, chemical and biological properties. Furthermore, foliar spray of humic acid facilitates greater uptake of nutrients which leads to the effective conversion of vegetative phase to flowering phase. These results are in conformity with the findings of Gawade *et al*. (2019) in Chrysanthemum and Bisinupada *et al*., (2020) in Gerbera . Supply of plant nutrients in different ways at different concentrations also differed significantly.

FLOWERE YIELD AND QUALITY PARAMETERS

The demand of gerbera as cut flower is more, so that production of gerbera mainly depends on the nutrient status of the soil. Although gerbera can be grown almost in any growing media, appropriate environment, adequate moisture, balanced nutrients and proper attention were needed for proper growth and quality of the flowers. Gerbera is mostly cultivated under protected condition and requires proper nutrient management to produce quality flowers. Application of proper and balanced nutrients influences the crop directly on every phase of growth. Owing to the ever growing awareness towards biological inputs which are renewable in nature and their positive impacts on environment and the increased cost of inorganic inputs which are exhausting gradually due to over exploitation. With light of facts acquired minimal use of inorganic inputs along with biological inputs such as organic manures, biofertilizers and biostimulants will sustain the production and effective for growers. The yield parameters (Table 2) *viz.,* number of flowers plant^{-1} (29.44) and number of flowers m^{-1} (235.52) were recorded in (T_7) with the application of 75 percent RDF + FYM and CP each @ 5 kg m^{-2} + 20 g m^{-2} of *Azospirillum* and Phosphobacter + 2 percent of Humic acid at 30 days intervals after planting could be considered as the best treatment for the cultivation of Gerbera cv. Pink elegance under polyhouse condition. The increase in yield parameters may be due to appropriate nutrients supplied for its sufficient growth. Application of inorganic nutrients along with farmyard manure enhanced the yield. Application of Coir pith compost along major nutrients plays a vital role in better nitrogen fixation from atmosphere, better root proliferation, uptake of nutrients and water which leads to initiates maximum flower yield in gerbera plants. It is clear with foliar application of humic acid recorded maximum yield (T_7) as compared to control.

Performance of the crop with respect to flower yield parameters is much important for crop like gerbera as they are economically valued for their uses. A perusal of the data indicated with foliar application of humic acid @ 2 percent along with appropriate quantity of organic and inorganic nutrients remarkably taken maximum yield of gerbera were observed when compare to other treatments. Significantly minimum yield were registered in control (T_1). Among all treatments, T_1 with the application 100 percent of recommended dose of fertilizer recorded the lowest flower yield of (144.88 m^{-2}) respectively. Similar results have been reported by Kishan Swaroop *et al*., (2017) in Gladiolus and Sendhilnathan *et al*.,(2020) in Carnation cv. White liberty. Quality of cut flowers are determined by the evaluating its physical characters such as diameter of flower, length of ray florets , length of flower stalk and diameter of flower stalk along with its ability to prolong the vase life. Gerbera cv. Pink elegance treated with T7 (75 percent recommended dose of fertilizer along with farm yard manure and coirpith compost each @ 5 kg m $^{-2}$ + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Humic acid @ 2 %) produced flowers with superior floral characters such as maximum flower diameter (10.15 cm), maximum length of ray florets (3.96 cm) , flowers with longer flower stalk (50.10 cm) and increased flower stalk diameter (0.491 cm) . This may be due to the effect of integrated nutrients which is supplied in different forms and appropriate quantity influenced on gerbera floral attributes, the increases in diameter of flower stalks and capitulum diameter due to the inoculation might be attributed to the biological fixation of nitrogen and solubilization of phosphorus in root parts of plants resulting in absorption of more nutrients and its utilization. Moreover, Azospirillum had a role in nitrogen fixation and also involved in the production of indole-3- acetic acid (IAA), gibberellic acid (GA) and cytokinin like substances which enhanced the growth of plants, phosphorous solubilizing bacteria helped in solubilization and mobilization of phosphorous in soil. The differential changes among various treatments in diameter of flower and length of flower stalk are shown in the (Table 1) Biofertilizers utilize certain microorganisms. These microorganisms trap atmospheric nitrogen and convert it into nitrates and nitrites and make it available to the plants. They also convert insoluble phosphates into the forms required by the plants. Humic acid is a commercial product which is produced by decaying organic compounds. It influences the plant growth and modifying the physiology of plants. The effect of

humic acid significantly increased capitulum diameters and floral characters compared to control. The biofertilizers, used for this experiment not only enhanced the efficiency of fertilizers but also partly supply nutrients, like fixing atmospheric N in a free living state by Azospirillum, these bacteria secrete some growth promoting factors, e.g. gibberellin, auxins and cytokinin-like substances. In addition to these traditional approaches, biostimulants have been highlighted as a promoter of optimizing productivity by modifying physiological processes in plants. Biostimulants offers a potentillay novel approach for the regulation and or modifications of physiological processes in plants to stimulate growth, to mitigate stress-induced limitations, and to increase flower yield and quality. Pre harvest factors influence the post harvest survival of cut flowers greatly. The integrated nutrient combination of 75 percent recommended dose of fertilizer along with farm yard manure and coirpith compost each @ 5 kg m^{-2}, *Azospirillum* and phosphobacter each @ 20 g m^{-2} along with humic acid @ 2 percent foliar application has a significant effect on vase life with maximum of 15.97 days. Concept of Integrated Nutrient Management is regulated nutrient supply for optimum crop growth and higher productivity, improvement and maintenance of soil fertility. Application of integrated nutrients at different intervals was achieved with longer stalk length and excessive accumulation of sugars in the stem, which might have translocated to corolla, thus increasing the water uptake and maintaining turgidity in the stem, resulting in prolonged shelf life of the flower. Biofertilizers contain cytokinins and auxin that might have increased the antioxidant levels and resistance to senescence. The increased vase life and shelf life might be due to by triggering of such metabolic activity and narrowing of the C : N ratio by the significant accumulation of carbohydrates. Application of biostimulants seems to encourage the development of quality parameters. This is due to the probable reasons for increased morphological characters by the cumulative effect of foliar application of humic acid which ultimately lead to enhanced cell division and cell enlargement, promotion of protein synthesis coupled with higher dry matter accumulation in the plant. Better quality of cut flowers produced as a result of application of biostimulants which is composed of cytokinin, indole-butryic acid and gibberellic acid. Similar results were revealed by Sathyanarayana *et al*.,(2018) in Gladiolus cv.American beauty and Kumar *et al*.,(2013) in Gladiolus cv. white prosperity.

CONCLUSION

Based on the above facts and results of the present studies on the influence of integrated nutrients on flowering, flower yield and quality of Gerbera (*Gerbera jamesonii)* it can be concluded that application of 75 percent recommended dose of fertilizer along with farmyard manure and coirpith each @ 5 kg m^{-2} + 20 g m^{-2} of *Azospirillum* and Phosphobacter + 2 percent of Humic acid at 30 days intervals after planting could be considered as the best treatment for the maximizing flowering, yield and quality of Gerbera cv. Pink elegance under poly house condition.

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Table 1: Treatment details	
`T_1	RDF 100% (control)
T_2	RDF 100% + *Azospirillum* and Phosphobacter each @ 20 g m^{-2}
T_3	T_2 + foliar spray of Humic acid @ 2%
T_4	T_2 + foliar spray of Panchgavya @ 2%
T_5	T_2 + foliar spray of Sea Weed Extract @ 2%
T_6	T_2 + foliar spray of Vermiwash @ 2%
T_7	RDF 75% + FYM and CP each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Humic acid @ 2%
T_8	RDF 50% + VC @ 2.5 kg m-2 and CP @ 5 kg m^{-2}+ *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Humic acid @ 2%
T_9	RDF 75% + + FYM and CP each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Panchgavya @ 2%
T_{10}	RDF 50% + VC @ 2.5 kg m-2 and CP @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Panchgavya @ 2%
T_{11}	RDF 75% + FYM and CP each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Sea weed extract @ 2%
T_{12}	RDF 50% + VC @ 2.5 kg m-2 and CP @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Sea weed extract @ 2%
T_{13}	RDF 75% + FYM and CP each @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2} + Vermiwash @ 2%
T_{14}	RDF 50% + VC @ 2.5 kg m-2 and CP @ 5 kg m^{-2} + *Azospirillum* and Phosphobacter each @ 20 g m^{-2}+ Vermiwash @ 2%

Table 2: Effect of Integrated Nutrients on Flowering of Gerbera (*Gerbera jamesonii)* Under Poly House Condition

Treatments	Days taken for emergence of flower bud	Days to full bloom of flowers	Days taken for fifty percent of flowering	Diameter of flowers (cm)	Length of ray florets (cm)	Length of flower stalk (cm)	Diameter of flower stalk (cm)
`T_1	28.61	38.66	14.97	8.15	3.02	38.98	0.397
T_2	26.09	36.12	13.29	9.13	3.49	49.29	0.457
T_3	24.87	34.95	12.63	9.59	3.72	49.70	0.477

T_4	25.21	35.36	12.93	9.33	3.63	49.59	0.469
T_5	24.62	34.41	12.58	9.51	3.70	49.67	0.478
T_6	25.71	35.87	13.14	9.23	3.57	49.43	0.461
T_7	23.81	33.98	11.94	10.15	3.96	50.10	0.491
T_8	26.84	36.41	13.71	9.05	3.41	49.21	0.491
T_9	23.98	33.64	12.07	10.03	3.87	49.98	0.459
T_{10}	27.29	37.52	13.98	8.92	3.35	49.11	0.488
T_{11}	24.13	34.62	12.35	9.79	3.79	49.79	0.451
T_{12}	28.19	38.45	14.51	8.65	3.21	49.08	0.481
T_{13}	24.27	34..11	12.29	9.84	3.80	49.87	0.443
T_{14}	27.78	37.10	14.18	8.79	3.27	49.05	0.488
S.ED	0.16	0.18	0.10	0.6	0.03	0.04	0.03
CD(P=0.05)	0.34	0.36	0.21	0.12	0.06	0.08	0.06

Table 3: Effect of Integrated Nutrients on Flower Yield and Quality of Gerbera (*Gerbera jamesonii)* Under Poly House Condition

Treatments	Number of flowers plant^{-1}	Number of flowers m^{-2}	Vase life (days)
`T_1	18.11	144.88	11.54
T_2	25.69	205.52	13.65
T_3	26.57	212.56	14.95
T_4	25.45	208.60	14.51
T_5	26.48	211.84	14.90
T_6	24.52	196.16	13.98
T_7	29.44	235.52	15.97
T_8	23.87	190.96	13.27
T_9	28.31	226..48	15.63
T_{10}	23.01	184.08	12.93
T_{11}	27.39	219.12	15.29
T_{12}	21.39	171.12	12.08
T_{13}	27.45	219.60	15.26
T_{14}	22.18	177.44	12.58
S.ED	0.40	3.2	0.15
CD(P=0.05)	0.80	6.4	0.31

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RESEARCH ARTICLE

Effect of Organic Manures and Natural Growth Stimulants on the Growth and Quality Characters of Bhendi

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ABSTRACT

The present study was carried out to establish the effect of organic manures along with natural growth stimulants on the growth and quality of bhendi. In order to study the effects of bulky organic manures and growth stimulants on bhendi, a field experiment was carried out in the farmer's field at P. Chettihalli village, Dharmapuri district, during January – April 2018. Ten treatments were replicated thrice using randomized block design. The treatments details are T_1- control (no treatment), T_2- 100% recommended dose of NPK, T_3- FYM @ 40 t ha^{-1}, T_4- pressmud@ 40 t ha^{-1}, T_5- FYM @ 40 t ha^{-1}+ foliar application of panchagavya (3%), T_6- FYM @ 40 t ha^{-1}+ foliar application of vermiwash (10%), T_7- FYM @ 40 t ha^{-1}+ foliar application of humic acid (0.3%), T_8- pressmud@ 40 t ha^{-1}+ foliar application of panchagavya (3%), T_9- pressmud@ 40 t ha^{-1}+ foliar application of vermiwash (10%) and T_{10}- pressmud@ 40 t ha^{-1}+ foliar application of humic acid (0.3%). Soil application of pressmud @ 40 t ha^{-1} along with foliar application of humic acid (T_{10}), recorded significantly highest value in growth characters like plant height (116.3 cm), number of leaves plant^{-1} (32.6), number of branches plant^{-1} (6.0), stem grith (3.72), leaf area index (6.92) and chlorophyll content (3.10 mg g^{-1}) and also the quality characters of bhendi was also improved by the application of bulky organic manures, the treatment T_{10} recorded the highest relative water content (85.4 per cent), ascorbic acid (15.42 mg 100g^{-1}), crude protein (1.65 per cent), total soluble solids (5.23 brix percent), titrable acidity (0.15 per cent) and low fibre content (9.85 per cent). The results of the field experiment proved that application of bulky organic manures (FYM and Pressmud) at higher dose (40 t ha^{-1}) significantly increased the growth characters (plant height, number of leaves plant^{-1}, number of branches plant^{-1} stem grith and leaf area index) and the quality characters (highest relative water content, ascorbic acid, crude protein, total soluble solids, titrable acidity and low fibre content). The untreated control treatment registered the lowest growth and quality characters of bhendi.

Keywords: Organic manures, natural growth stimulants, growth and quality.

INTRODUCTION

Bhendi (*Abelmoschus esculentus L,*) is native of tropical Africa. Out of 34 species of *Abelmoschus*, only the species, *Abelmoschus esculentus* is known to be cultivated extensively as commercial vegetable. Bhendi (*Abelmoschus esculentus)* is one of the popular vegetable in India. It is a fast growing annual which has captured a prominent position among the vegetables in India. It is a multiple use crop, grown in all agro-ecological zones of India mainly for its immature fruits which are eaten as cooked vegetable. It is widely cultivated in India in the states of Uttar Pradesh, Assam, Bihar, Orissa, Maharastra, West Bengal, Karnataka, Tamilnadu and Andhra Pradesh. Bhendi, being a short duration vegetable crop, its growth, yield and quality are largely influenced by the application of nutrient through different sources. It requires proper and sufficient supply of all essential plant nutrients for regular fruiting and subsequent pickings (Premsekhar and Rajashree, 2009). Farming with organic manures gains potential importance because it is claimed that the crops grown with organics are free from pesticides and chemical residues, taste well and are morenutritious, thereby increasing export potential (Prabhu *et al*., 2003). Manures are usually applied at higher rates, relative to inorganic fertilizers, and they give residual effects on the growth and yield of succeeding crops (Makinde and Ayoola, 2012). Organic manures constitute a source of macro and micro nutrients and are helpful in improving physical, chemical and biological health of soil, reduces nutrient losses, increases nutrient availability and uptake, produces harmful residues free produce, improve the quality of vegetables (Acharya and Mandal, 2000) and (Tiamiyu *et al*., 2012).

Farm Yard Manure is a bulky organic manure, as well as a good soil conditioner it is probably the best and safest of all manures, natural or artificial. The quantities of farmyard manure necessary to keep a soil in a fertile condition vary according to the soil and its nature. Farmyard manure has been used as a soil conditioner since ancient times and its benefit have not been fully harnessed due to large quantities required in order to satisfy the nutritional needs to crops (Makinde *et al*., 2007). Incessant land application of pressmud cake to farming crops for 5–6 years is likely to improve soil health by adding sulphur (S) and organic matter to soil (Razzaq, 2001). Pressmud is reported to be a valuable resource of plant nutrients and may therefore improve physical, chemical and biological properties of a soil (Rangaraj *et al.,* 2007). Interestingly, panchagavya had the highest population of total bacteria, actinomycetes, phosphate solubilizers, fluorescent pseudomonas and nitrifiers. In addition, dehydrogenase activity and microbial biomass carbon were also found to be higher in panchagavya (Amalraj *et al.,* 2013). Vermiwash contains 0.5% N, 0.39% P and 0.46% K (Jasmin, 1999). The assessment of vermiwash indicated the presence of micronutrients in significant quantity (Ismail, 2005). Application of vermicompost and vermiwash along with recommended dose of NPK inorganic fertilizers or alone improves the yield and yield contributing parameters of bhendi (Kulkarni *et al.,* 2004 and Paramasivan *et al.*, 2006), due to its growth promoting activity (Rajan and Murugesan, 2012 and Nath and Singh, 2012). Researchers explained the beneficial effects of humic acid such as increasing cell membrane permeability (Sial *et al*., 2007). The significance of humic acid is not limited to their hormone like activity it also improves stress tolerance (Yildirim, 2007), with this background.

MATERIALS AND METHODS

A field experiment was conducted in a farmer's field located at P. Chettihalli village near Palacode taluk, Dharmapuri district, Tamilnadu during January to April 2018, to establish the effect of soil application of organic manures and foliar fertilization of natural growth stimulants on the growth, and quality attributes of bhendi crop. The experimental soil was sandy loam in texture with pH of 7.40, electrical conductivity of 0.14 d Sm^{-1} and organic carbon 4.82 kg ha^{-1}. The available nitrogen, phosphorus and potassium content of the soil were 174.8, 18.7 and 93.6 kg ha^{-1} respectively. The exchangeable calcium, magnesium content was 7.5, 3.2 c mol (p+) kg^{-1} and available sulphur content of the soil was 7.4 mg kg^{-1}. The DTPA extractable Zn, Cu, Mn and Fe was 0.398, 1.15, 1.09 and 4.85 mg kg^{-1}, respectively. The field experiment was conducted in Randomized Block Design with ten treatments. Each treatment was replicated thrice. The treatments details are, T_1- Control (no treatment), T_2 - 100% Recommended dose of NPK, T_3- FYM @ 40 t ha^{-1}, T_4 - Pressmud@ 40 t ha^{-1},T_5 - FYM @ 40 t ha^{-1}+ foliar application of Panchagavya (3%), T_6 - FYM @

40 t ha^{-1}+ foliar application of Vermiwash (10%), T_7 - FYM @ 40 t ha^{-1}+ foliar application of Humic acid (0.3%), T_8 - Pressmud@ 40 t ha^{-1}+ foliar application of Panchagavya (3%), T_9 - Pressmud@ 40 t ha^{-1}+ foliar application of Vermiwash (10%), T_{10} - Pressmud@ 40 t ha^{-1}+ foliar application of Humic acid (0.3%). The calculated quantity of fertilizer was applied to the treatment (T_2) through urea, single super phosphate and muriate of potash. Half dose of (50 percent) N and full dose of P and K were applied basally and remaining 50 percent of N was applied at first weeding. The Bhendi hybrid siva was grown as test crop. Seeds were dibbled with spacing of 45×30 cm. The foliar application of natural growth stimulants *viz.,* humic acid (0.3%), vermiwash (10%) and panchagavya (3%) were applied thrice on 30, 60 and 90 DAS. Usual cultural operations were followed and crop was allowed to grow up to harvest. Five plants were selected from each plot to record the biometric observations of growth characters (plant height, number of leaves plant^{-1}, number of branches plant^{-1} stem grith and leaf area index) and the quality characters (highest relative water content, ascorbic acid, crude protein, total soluble solids, titrable acidity and low fibre content).

RESULT AND DISCUSSION

Growth characters

Plant height

The soil application of pressmud @ 40 t ha^{-1} and foliar application of humic acid (0.3%) [T_{10}] recorded the highest plant height of 116.3 cm. However, the treatment [T_{10}] was found to be on par with [T_9 - pressmud @ 40 t ha^{-1} and foliar application of vermiwash] and [T_8 - pressmud @ 40 t ha^{-1} and foliar application of panchagavya] (Table 1). The results of the study clearly indicated that bhendi responded well for soil application of organic manures and foliar application of growth stimulants. The primary goal of organic farming is to optimize the health and productivity of interdependent communities of soil life, plants, animals and people (Yuda *et al*., 2016). The growth components of bhendi were significantly increased by application of organic manures. Moreover, the humic acid acts as growth regulators and by retard the activity of IAA oxidase in the plant system and pro long the persistence of IAA in plants increased the cell division and cell enlargement which led to increased plant height (Dhanasekaran *et al.,* 2007).

Number of branches plant^{-1}

The soil application of pressmud @ 40 t ha^{-1} along with humic acid at 0.3% [T_{10}] recorded the highest number of branches plant^{-1} (5.98). This was followed by the treatment applied with pressmud @ 40 t ha^{-1} along with vermiwash at 10% [T_9] (5.46) and pressmud @ 40 t ha^{-1} along with panchagavya at 3% [T_8] (5.18) (Table 1). However, the treatment T_{10} was found to be on par with T_9 and T_8. The results clearly showed that application of growth stimulants along with press mud recorded significantly higher number of branches as compared to the treatment supplied with T_4 pressmud @ 40 t ha^{-1} alone. The increased number of branches plant^{-1} in bhendi due to the supply of nutrients through organic sources was reported by Suchithra and Manivannan (2012). Similar results were obtained by Muhammad and Khattak (2009).

No. of leaves plant $^{-1}$

Among the three growth stimulants tried with pressmud, foliar feeding of humic acid to pressmud [T_{10}] applied plant excelled the other two treatments T_8 [pressmud @ 40 t ha^{-1} and foliar application of panchagavya] and T_9 [pressmud @ 40 t ha^{-1} and foliar application of vermiwash] by recording the higher number of leaves plant^{-1} of 32.6 (Table 1). However, the treatment T_8 and T_9 was found to be on par with T_{10}, similar trend was noticed with FYM also. The enlargement in cell size and cell division by increasing nutrients from FYM might have helped in growth parameters like plant height and number of leaves. These results are in agreement with those reports of Anburani and Manivannan (2002). Humic acid applied through foliage appeared to behave like a plant growth regulator and helped to produce more number of leaves plant^{-1}. Similar results were also given by Abed (2016).

Stem grith

Soil application of pressmud @ 40 t ha^{-1} and foliar feeding of humic acid recorded the highest stem grith of 3.72 cm (Table 1). Soil application of pressmud increased the availability of N in soil. Nitrogen plays an important role in the synthesis of chlorophyll and amino acid which contributes to build units of protein and thus growth of plants. Further, the growth and development of plant mostly depends upon differentiation and expansion of cell component. Increase in shoot grith with vermiwash treated plants might be due to supplementation of essential nutrients through foliar application of vermiwash (Rajan and Murugesan, 2012).

Leaf area index

Among the three growth stimulants tried with pressmud, T_{10} humic acid by recording the highest leaf area index of 6.92 (Table 1). However, the treatments T_8 and T_9 were found on par with T_{10}. Photosynthetic capacity of plants is a function of photosynthetically active leaf area which is indicated by LAI. Patil *et al.*, 2004 reported that the leaf area index was significantly higher with application of FYM, which might be due to production of more capsicum leaf area through higher nutrients uptake of both macro and micronutrients resulting in balanced nutrition. Increased leaf area implies higher light interception and dry matter production which invariably promotes the plant growth (Chen *et al*., 2004).The increased production of auxin and growth substances by humic acid at early phase of growth would have increased the leaf area in bhendi (Ballal Anand and Kadam 2016).

Chlorophyll content

The treatment which received pressmud @ 40 t ha^{-1} along with foliar application of humic acid [T_{10}] recorded the highest chlorophyll content of 3.10 mg g^{-1} followed by the treatment [T_9] supplied with pressmud @ 40 t ha^{-1} and vermiwash, which recorded the chlorophyll content of 3.05 mg g^{-1}and the treatment T_8 recorded the chlorophyll content of 3.03 mg g^{-1} (Table 1). However, the treatments T_8 and T_9 was found to be on par with T_{10}, similar trend was noticed with FYM also. The IAA is the component of various enzymes, such as carbonic anhydrase and alcoholic dehydrogenase, which have a suggestive role in chlorophyll formation, photosynthesis and metabolic reactions in plants (Venkataramana, 2002). Increased growth of bhendi with the foliar application of humic acids might be due to increased rate of photosynthesis and respiration contributed by the protein and quinone groups of the assimilated humic acid (Sanwal *et al.,* 2007). The improvement in the persistence of IAA in the plant tissue due to the foliar application of humic acid might have helped for chlorophyll formation; similar result was reported by Halime Ozamar Unlu *et al.* (2011) in cucumber.

Quality characters

Relative water content and Total soluble solids

Among the treatments, soil application of pressmud @ 40 t ha^{-1} and foliar application of humic acid [T_{10}] recorded the highest relative water content of 85.4 percent. However, the treatment T_{10} was found to be on par with T_9 [pressmud @ 40 t ha^{-1} and foliar application of vermiwash (84.7 percent)] and T_8 [pressmud @ 40 t ha^{-1} and foliar application of panchagavya (83.6 percent)]. Among the three growth stimulants tried with pressmud, foliar application of humic acid [T_{10}] out-performed vermiwash [T_9] and panchagavya [T_8] by recording the highest total soluble solids of 5.23 % Brix (Table 2). Zayas *et al.,* (2018) evaluated the effect of humic acid on growth and quality of tomato. They observed higher growth rate, and vigor in humic acid treated plants than the control. They also reported that humic acids positively increased several quality parameters of tomato fruit like pH, total soluble solids and total soluble carbohydrates.

Ascorbic acid content

The soil application of pressmud @ 40 t ha^{-1} and foliar application of humic acid [T_{10}] recorded the highest ascorbic acid content of 15.42 mg 100 g^{-1}. However, the treatments T_9 - [pressmud @ 40 t ha^{-1} and foliar application of vermiwash (15.21 mg 100 g^{-1})] and T_8 -[pressmud @ 40 t ha^{-1} and foliar application of panchagavya (14.75 mg 100 g^{-1})] were found to on par the treatment T_{10} (Table 2).The increase in ascorbic acid content might be ascribed to better availability and uptake of plant required nutrients and also favourable soil conditions developed by the applied FYM, which help in the synthesis of chlorophyll and increased ascorbic acid content (Patil *et al.,* 2004). Increase

ascorbic acid content due to application of organic manures was also reported by Shashidhara (2000) in capsicum fruits and Sable *et al.* (2007) in tomato.

Crude protein content

Among the three natural growth stimulants tried with pressmud, foliar feeding of humic acid to pressmud [T_{10}] applied plant excelled the other two treatments T_8 [pressmud @ 40 t ha^{-1} and foliar application of panchagavya (14.1 percent)] and T_9 [pressmud @ 40 t ha^{-1} and foliar application of vermiwash (14.3 percent)] by recording the highest crude protein content of 14.5 percent T_{10} (Table 2). However, the treatments T_8 and T_9 was found to be on par with T_{10}, similar trend was noticed with FYM also. The higher crude protein content in these treatments could be attributed to improved uptake of N from soil (Rani and Jose, 2009).

Crude fibre content

The treatment T_{10} [pressmud @ 40 t ha^{-1} along with humic acid] recorded lowest the crude fibre content of 9.85 percent. However, the treatment [T_{10}] was found to be on par with T_9 - [pressmud @ 40 t ha^{-1} along with vermiwash (9.95 percent)] and T_8 - [pressmud @ 40 t ha^{-1} along with panchagavya (10.17 percent)] (Table 2).The decrease in crude fibre content due to the increase in succulence by application of organic manures was reported by Hisham *et al.* (2014).

Titrable acidity

The soil application of pressmud @ 40 t ha^{-1} along with foliar application of the natural growth stimulant humic acid [T_{10}] recorded the lowest titrable acidity of 0.15 per cent (Table 2). However, the treatments T_8 – [pressmud @ 40 t ha^{-1} along with foliar application of panchagavya (0.15 per cent)] and T_9 – [pressmud @ 40 t ha^{-1} along with foliar application of vermiwash (0.16 per cent)] were found to be on par with [T_{10}]. Zayas *et al.,* (2018) evaluated the effect of humic acid on growth and quality of tomato the positively influenced the titrable acidity of fruit.

CONCLUSION

From the results of the study, it was concluded that application of bulky organic manures at higher dose (40 t ha^{-1}) and foliar application of natural growth stimulants increased its performance over sole application of organic manures alone. The treatment supplied with 40 tons of pressmud had showed better compared to other treatments on growth and quality characters of bhandi. On doubling the doses to meet out the nutrient requirement to the crop was showed an positive results on bhendi crop.

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Table 1: Effect of organic manures and foliar fertilization of natural growth stimulants on growth characters of bhendi

Treatment details	Plant height (cm)	No. of branches plant $^{-1}$	No. of leaves plant $^{-1}$	Stem grith (cm)	Leaf area index	Chlorophyll content (mg g^{-1})
T_1 – Control	79.0	3.0	24.0	2.49	4.51	1.61
T_2 - NPK alone	84.4	3.3	25.4	2.71	4.98	2.11
T_3 - FYM 40 t ha^{-1}	90.6	3.5	26.8	2.90	5.34	2.26
T_4 - Pressmud 40 t ha^{-1}	105.2	4.9	30.2	3.41	6.32	2.82
T_5 - FYM 40 t ha^{-1}+ Panchagavya	96.2	3.7	28.2	3.08	5.66	2.47
T_6 - FYM 40 t ha^{-1}+ Vermiwash	97.6	4.2	28.5	3.12	5.71	2.51
T_7 - FYM 40 t ha^{-1}+ Humic acid	98.4	4.7	29.0	3.20	5.92	2.57
T_8 - Pressmud 40 t ha^{-1}+ Panchagavya	111.9	5.1	31.5	3.60	6.66	3.03
T_9 - Pressmud 40 t ha^{-1}+ Vermiwash	113.7	5.4	32.0	3.62	6.80	3.05
T_{10} - Pressmud 40 t ha^{-1}+ Humic acid	116.3	6.0	32.6	3.72	6.92	3.10
SE$_d$	**2.55**	**0.08**	**0.55**	**0.076**	**0.142**	**0.052**
CD (p=0.05)	**5.36**	**0.17**	**1.16**	**0.160**	**0.30**	**0.110**

Table 2: Effect of organic manures and foliar fertilization of natural growth stimulants on quality characters of bhendi

Treatment details	Relative water content (%)	Total soluble solids (% Brix)	Ascorbic acid content (mg 100 g^{-1})	Crude protein (%)	Crude fibre (%)	Titrable acidity (%)
T_1 – Control	63.0	3.34	10.12	9.3	13.92	0.31
T_2 - NPK alone	66.8	3.71	11.10	10.0	13.17	0.28
T_3 - FYM 40 t ha^{-1}	70.4	4.10	11.82	10.6	12.52	0.25
T_4 - Pressmud 40 t ha^{-1}	79.3	4.82	13.97	13.0	10.89	0.19
T_5 - FYM 40 t ha^{-1}+ Panchagavya	74.1	4.39	12.59	11.6	11.90	0.23
T_6 - FYM 40 t ha^{-1}+ Vermiwash	74.9	4.46	12.70	11.8	11.75	0.22
T_7 - FYM 40 t ha^{-1}+ Humic acid	75.5	4.51	13.22	12.1	11.63	0.22
T_8 - Pressmud 40 t ha^{-1}+ Panchagavya	83.6	5.13	14.75	14.1	10.17	0.16
T_9 - Pressmud 40 t ha^{-1}+ Vermiwash	84.7	5.18	15.21	14.3	9.95	0.15
T_{10} - Pressmud 40 t ha^{-1}+ Humic acid	85.4	5.23	15.42	14.5	9.85	0.15
SE$_d$	**1.51**	**0.104**	**0.33**	**0.33**	**0.29**	**0.0095**
CD (p=0.05)	**3.61**	**0.220**	**0.71**	**0.70**	**0.62**	**0.0201**

RESEARCH ARTICLE

Quality by Design Approach in Natural Product Extraction

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ABSTRACT

Extraction of active constituents from plant products is a very essential and tedious process and gives more important active components present in the plant. Standard extraction of natural products may cause loss of product quality, some important active constituents, low yield, and more solvent consumption. In that case, one should search for alternative approaches. The right choice was recently developed quality by design approach. During the natural product extraction, to reduce these problems and it is simple to select the extraction method and type of solvent used, the time and the temperature to be maintained. Also, the quality by design is an approach that is cost-effective and gives more yield. Here in this design different extraction methods for extraction of different plants and the yield obtained and other parameters are studied. Based on the reviews, the central composite design in QbD was selected and concluded parameters to be selected for high yield which has a high amount of organic phase and time gives more yields.

Keywords: Extraction, Quality by Design, central composite design.

INTRODUCTION

Extraction of active constituents from plant products is a very essential and tedious process as one should know the important active constituents present in the plant and should have an idea about the components that may be present without basic knowledge of the active constituents it is difficult to select the extraction method, solvent to be used, the temperature used and time required for extraction. Hence it is better if one has a basic knowledge of the extraction methods for various plants and method is suitable for polar/non-volatile constituents and which method is suitable for nonpolar/ volatile constituents. Based on this basic knowledge one can apply the QbD approach and then it is simple to select the extraction method and type of solvents used and time and temperature to be maintained etc.

Extraction

The initial stage in obtaining desired natural compounds from raw sources is extraction. Distillation, Solvent extraction, sublimation, and pressing are some of the extraction techniques based on the principle of extraction. The most widely used approach is solvent extraction. The natural product extraction involves the following steps: [1]the solvent diffuses through a solid substrate [2] solute liquefies in the solvents [3] the extracted solutes diffuse out of the solid matrix [4]collected are the extracted solutes. [1-5]

Maceration

Although maceration is a simple extraction procedure, it has the disadvantages of a lengthy extraction process and poor extraction efficiency. Thermolabile compounds may be extracted [6].

Percolation

Percolation is a continuous procedure that is more effective than maceration in that the saturated solvent is continuously replaced by a new solvent [7].

Decoction

A decoction is a technique for extracting tough materials like roots and bark. The decoction extract comprises a large number of water-soluble pollutants. The decoction can't be utilized to extract thermolabile or volatile components[8].

Reflux extraction

Percolation or maceration is less effective than reflux extraction, and it takes less time and less solvent. This method is ineffective for extracting thermolabile natural compounds [9].

Soxhlet extraction

The Soxhlet extraction technique uses the concepts of reflux and syphoning to continuously extract the herb with a new solvent, combining the advantages of percolation and reflux[10].

SFC (Supercritical Fluid Chromatography)

In SFC, the mobile phase is a supercritical fluid. SFC combines the benefits of gas chromatography as well as liquid chromatography[11].

MAE (Microwave-Assisted extraction)

By interacting with polar plant materials including water and certain chemical molecules, microwaves generate heat [12-13].

PLE (Pressurized liquid extraction)

According to different research organizations, PLE is often called pressurized fluid extraction, enhanced solvent extraction, high-pressure solvent extraction, and accelerated fluid extraction. During the extraction, PLE exerts a lot of pressure [14-15].

QUALITY BY DESIGN (QbD)

Pharmaceutical research aims to create a high-quality product and production process that reliably produces the desired outcomes. Scientific knowledge is provided to assist the design space, needs, and production controls through pharmaceutical discovery research and production experience. Quality risk management might be based on information from pharmaceutical development research. It's critical to understand that products can't be tested for quality; instead, quality must be designed into the product. During development and lifecycle management, deviations in formulation and manufacturing processes should be considered as a chance to acquire additional knowledge and enhance the design space's establishment. Also, using important knowledge gathered from research that yielded unexpected findings can be beneficial. The applicant proposes a design space, which is focused on regulatory review and authorization. It's not rare for people to work in the design industry. It is nearly always necessary to undergo a regulatory post-approval modification procedure if move out of the design region. A product must be designed to meet both the demands of patients and the expected performance of the product in such conditions. Product development tactics vary from one company to the next, as well as from one product to the next. Quality risk management and knowledge management (ICH Q10) are adopted throughout the product's life cycle. This methodical approach can help a corporation achieve the desired quality of the product while also assisting regulators in well understanding the company's strategy [16-20].

ICH Q 8 (R1)

Clear goals are the first step in a systematic design strategy that focuses on product and process knowledge and control and is based on fundamental science and quality risk management [21].

FDA PAT Guidelines

A process for developing, evaluating, and monitoring production by measuring key quality and performance parameters of new and existing products and techniques in real-time (during processing) to ensure final product safety [22-23] .

Benefits of QbD [16, 18, 20, 21]

- Remove batch inconsistencies
- Decrease deviations and high studies
- Utilize QbD to avoid issues with regulatory compliance.
- A long-term speculation is organizational learning.
- Quality by Design is good science
- Better development conclusions
- Technical staff empowerment

Opportunities [22-23]

- Competent, agile, flexible system
- Reduce project failures and wastage by improving production efficiency and lowering costs.
- Better scientific knowledge of all products
- Good communication between industry and scientific concerns
- Accurate, reliable information
- Utilize risk management

APPLICATIONS OF QbD

- QbD method helps in the selection of a wide range of solvents
- QbD approach helps in improving the yield of active constituents in extraction
- QbD helps in the selection of different concentrations of solvent composition
- QbD helps in the extraction of selected active constituents based on their polarity/volatility
- Based on the researcher's available resources like solvent and time one can select an extraction procedure with the desired output.

- In a short period with limited available resources most efficient extraction is achievable with qbd

EXAMPLES OF EXTRACTION TECHNIQUE & YIELD OF SOME PLANTS

***T. chebula* fruit extraction and gallic acid isolation**

T. chebula fruits have been crushed into a powder form (100g), and 250mL of methanol was used in a Soxhlet extractor for a 15-hour extraction. The extraction yield was 25%.

Fruit Extract of *Elaeocarpus ganitrus*

Elaeocarpus ganitrus fruit extract was made using Soxhlet extraction procedures with 90 percent ethanol, 40 grams of powder, 200 milliliters of solvent, and 11.46 grams of extract. The percentage yield of crude extract was found to be 28.65 %.

Extract of *Ficus capensis* leaves

The solvents for the various extractions were *Ficus capensis* leaves treated with ethanol and water. The plants dried and powdered leaves (500g) were repeatedly extracted by cold maceration with 95 percent ethanol, with a crude extract yield of 30%.

Extraction of Dried katuk leaf

Ethanol was used as the solvent to produce katuk leaves in various quantities, including 50 percent, 70 percent, and 96 percent. Each extraction process lasted 24 hours. Cold maceration was used to extract dried katuk leaf powder (150.0 g), producing a 10% yield of extract.

***Senna tora* (L.) Seed Polysaccharide**

Solvent extraction is used to prepare *Seena tora* seeds. A benzene-ethanol solution was used to soak the powder (1: 1) *Seena tora* seeds (10 g) were soaked in 200 mL distilled water and agitated for 3-4 hours with an overhead stirrer. The extract has a 35 % w/w yield.

Green *Ginkgo biloba* leaves

Leaves of Ginkgo biloba prepared by supercritical fluid extraction with 70% ethanol used as a solvent for 2 h under reflux. 2.1% yield.

Tulsi Leaf extraction of *Ocimum sanctum*

O. sanctum leaves are crushed and extracted in chloroform, ethanol, methanol, as well asethyl acetate. A total of 20g of dried crushed leaves were steeped for 48 hours in 200 mL of solvent. The solvent was decanted, and the residue was steeped for another 24 hours in the same solvent. 6.25 % yield.

Flowers of *Hibiscus rosa sinensis* Linn

Soxhlet apparatus was filled with powdered flowers (500 g). For around 30-35 complete cycles, the medication was defatted using petroleum ether (60-80°C). In a Soxhlet device, defeated material was extracted using two litters of a 7:3 alcoholic: water mixture. Ethanolic extract yielded 7.6% w/w. All the above-reported extraction processes of those plants give fewer yields. The central composite design provides the percentage yield determination before the extraction of the plants commences by comparing the organic phase, time, and temperature. Table 2-4 gives A central composite analysis of the various parameters in order to get a high percentage yield.

RESULTS AND DISCUSSION

The yellow colour shows <10% of yield, the red colour shows 10-20% of yield, the white colour shows 30-40% of yield, and the temperature remains constant at 50°C, while the organic phase and time vary. The yellow colour shows <10% of yield, the red colour shows 10-20% of yield, the white colour shows 20-40% of yield, and the green

colour shows 40-50% of yield, the time remains constant at 30minutes, while the temperature and organic phase vary. The yellow colour shows <10% of yield, the red colour shows 10-20% of yield, the white colour shows 20-40% of yield, and the green colour shows 40-50% of yield, the organic phase remains constant at 65°C, while the temperature and time vary.

CONCLUSION

Based on the above QbD/DoE diagram it was concluded the high

The high amount of organic phase and high amount of time will give more yield. When the temperature is at (-2,-1) level that is 30-40% high yield is observed irrespective of the organic phase whereas time is kept constant at 30 minutes. Time at (-2) and temperature at (+2) there is more yield whereas the time increases and the temperature increases the yield decrease when the organic phase is kept constant at 65%. Hence the QbD approach is more helpful for pharmacognosy & phytochemistry or botanical scientists/researchers in terms of selection of extraction method and solvent selection as per their desired component interest.

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Table 1: Different extraction processes for natural products

Extraction process	Solvent	Time	Temperature	Volume of organic consumed	Polarity of naturally extracted products
Percolation	Water, non-aqueous and aqueous solvents	24hrs	32°C	more	Depending on extracted solvent
Maceration	Water, non-aqueous and aqueous solvents	72hrs	40-60°C	more	Depending on the extracted solvent
Decoction	Water	24hrs	32°C	none	Polar compounds
Soxhlet extraction	Organic solvents	24hrs	70°C	moderate	Depending on extracted solvent
Reflux extraction	non-aqueous and aqueous solvents	1-3hrs	60-80°C	moderate	Depending on extracting solvent
SFE	Supercritical fluid (usually s-co_2) occasionally with modifiers	Short time	31°C,300bar	less	Non-polar to moderate polar compounds
Microwave-assisted extraction	Water, non-aqueous and aqueous solvents	1-10minutes	65-100°C	less	Depending on the extracted solvent
Pressurized liquid extraction	Water, non-aqueous and aqueous solvents	Short time	200°C	less	Depending on extracting solvent

Table 2: Central composite design layout

S.No	Combination	X1	X2	X3	% Yield
1	I	50	20	40	25
2	X1	80	20	40	28
3	X2	50	40	40	30
4	X1X2	80	40	40	10
5	X3	50	20	60	35
6	X1X3	80	20	60	2
7	X2X3	50	40	60	6
8	X1X2X3	80	40	60	7
9	MIDPOINT	65	30	50	11
10	X1AT-2L	35	30	50	15
11	X1AT+2L	95	30	50	6
12	X2AT-2L	65	10	50	10
13	X2AT+2L	65	50	50	16
14	X3AT-2L	65	30	30	13
15	X3AT+2L	65	30	70	7

Note: X1-Organic Phase; X2-Time; X3- Temperature

Table 3: A central composite analysis

S.No	Combination	coefficient
1	B0	17.0
2	B1	-4.1875
3	B2	-1.5625
4	B3	-3.4375
5	B12	1.375
6	B13	-1.875
7	B23	-1.375
8	B11	-0.875
9	B22	-0.25
10	B33	-1.0

Table 4: Analysis of parameters

S.No	Combination	coefficient	F-valve	SS Ratio
1	B0	17.875	0.0	--
2	B1	-6.125	21.96	25.7204%
3	B2	-4.625	12.5216	14.6652%
4	B12	1.375	1.1064	1.2962%
5	B3	-5.375	16.9112	19.8072%
6	B13	-1.875	2.0576	2.4103%
7	B23	-1.375	1.1064	1.2962%

Note: Error variance: 13.6667; F standard valve at 0.1p:5.54
Standard deviation: 3.6968; F standard valve at 0.05p:10.1
Curvature effect: -7.375; F standard valve at 0.01p:34.1
95% confident levels of curvature effects from: -14.5765 To: -0.1735

Table 5: ANOVA Table

S.No	Src of variance	ss	DF	Ms	F-valve	Fstd at0.1p	Fstd at0.05p	Fstd at0.01p
1	Model	760.75	6	126.7917	0.3122	58.2	234	5859
2	Error	406.125	1	406.125				
3	Total	1166.875	7					

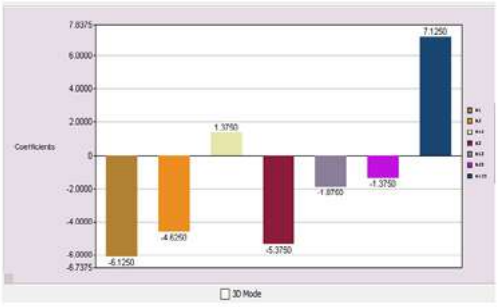

Figure 1: 3D Mode SS Ratio

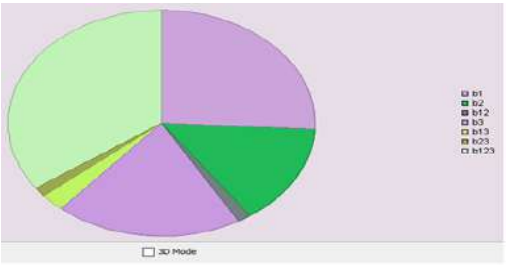

Figure 2: 3D Mode coefficient

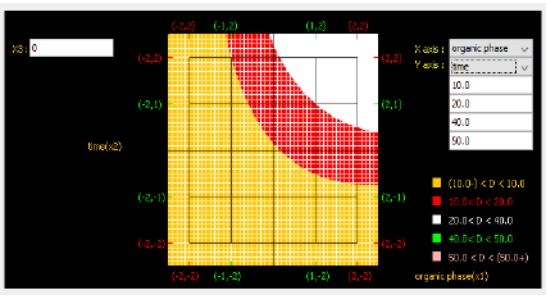

Figure 3: Yield comparison of X3

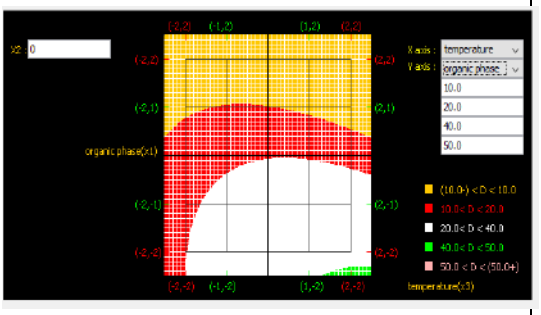

Figure 4:Yield comparison of X2

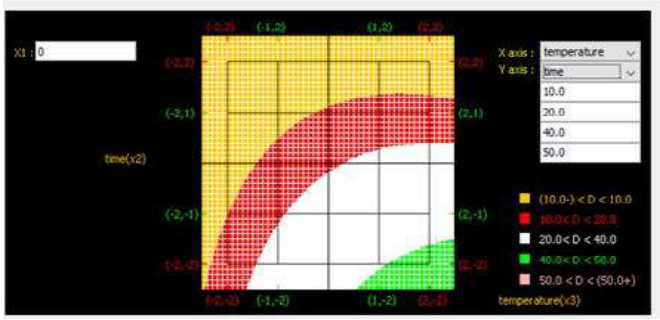

Figure 5:Yield comparison of X1

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RESEARCH ARTICLE

Impact of Walton's Dimensions on Studies of Quality of Work Life

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ABSTRACT

The paper presents literature on quality of work life (QWL) & seeks to relate it with Walton (1974) dimensions. The 8 dimensions as given by Walton (1974) are taken and various literature are reviewed, and a congruence is established between them. The paper poses questions to futureresearchers as to find new associations with Walton (1974) and other studies conducted on Quality of work life to take full usage of QWL by future businesses.

Keywords: Quality of Work life, Walton, dimensions, congruence, relationship, business

INTRODUCTION

The term quality of work life (QWL) poses different connotations. Sometimes it's considered as industrial democracy along with enhanced employee's say in decisions. In case of managers, it entails improvements in psychological aspects to advance outcome. Employees stress on equal sharing of profits, safe, secured, healthy and humane working atmosphere. Others propose improved social relationships by forming autonomous workgroups. Broadly it refers changing organizational climate through humanization of work, individualized organizations, and transitions in managerial and structural systems. Quality of work life relates with perception of favourableness or unfavourableness of job climate by employees. It denotes process of participative decision making & forming building mutual trust and respect between managers and employees. It concerns increased labour - management cooperation to resolve performance and worker satisfaction. Employees participate keenly and shape organizational atmosphere, methodology & results. This ensures goals of enhanced effectiveness for enterprise as well as perception of quality of work life by workers. Main aspects of Quality of Work Life as per Walton (1974) are – Adequate and Fair Compensation relate with balance between efforts and reward for employees. Safe and Healthy working conditions entail working hours, hygienic workplace and risk free environment Opportunity to use and develop human capacities include autonomy, control, performance feedback, skill exploitation and enhancement. Opportunity for career growth involve continual growth in career. Social Integration in the work force incorporate

openness, community, self esteem, upward mobility, unbiased treatment. Constitutionalising in the work organization stresses on constitutional protection, fair practices, due process, free speech. Work and personal life embraces balancing work and personal life. Social relevance of work lay emphasis on social cause. This paper presents current literature on QWL and seeks to relate with Walton (1974) dimensions. The 8 dimensions given by Walton (1974) are taken and various literature are reviewed, and a congruence is established between them.

LITERATURE REVIEW

Walton' dimensions and Further Studies on Quality of Work Life

Main aspects of Quality of Work Life as per Walton (1974) are –

Adequate and Fair Compensation – Balance between efforts and reward for employees. - Nanjundeswaraswamy and Swamy (2013); Royuela et al. (2008); Baleghizadeh and Gordani (2012); Noor and Abdullah (2012); Louis (1998); Tabassum (2012); Saraji & Dargahi (2006); Royuela et al. (2009); Jabeen et al. (2018); Lee et al. (2015); Normala (2010); Nowrouzi et al. (2016); Lee, et al. (2007); Chan et al. (2007); Vagharseyyedin et al. (2011); Sirgy et al. (2001); Almalki et al. (2012); Bujang (2010); Koonmee et al. (2010); Fernandes et al. (2017); Daniel (2019); Rosser & Javinar (2003); Allam & Shaik (2020); Allam & Shaik (2020); Daniel (2019); Moradi et al. (2014); Tabassum et al. (2011); Cacioppe & Mock (1984); Brooks & Anderson (2005); Rosser & Javinar (2003); Alserhan et al. (2021); Brooks et al. (2007); Rahimiyan & Najafi (2015); Mosadeghrad (2013); Al Kuwaiti & Subbarayalu (2019).

Safe and Healthy working conditions – Working hours, hygienic work place and risk free environment - Nanjundeswaraswamy and Swamy (2013); Elizur and Shye (1990); Bagtasos, M. R. (2011); Beh and Idris (2006); Arif and Ilyas (2013); Royuela et al. (2008); Da Silva Timossi et al. (2008); Rossmiller (1992); Narehan et al. (2014); Lee et al. (2013); Baleghizadeh and Gordani (2012); Noor and Abdullah (2012); Farid et al. (2015); Louis (1998); Tabassum (2012); Bragard et al. (2015); Saraji & Dargahi (2006); Wan & Chan (2013); Royuela et al. (2009); Jabeen et al. (2018); Lee et al. (2015); Normala (2010); Nowrouzi et al. (2016); Kanten & Sadullah (2012); Almalki et al. (2012); Lee, et al. (2007); An et al. (2011); Chan et al. (2007); Vagharseyyedin et al. (2011); Al-Qutop & Harrim (2011); Cohen et al. (1997); Sirgy et al. (2001); Kraut et al. (1989); Jorde Bloom (1996); Rathi (2009); Hart (1994); Almalki et al. (2012); Eaton et al. (1992); Bujang (2010); Koonmee et al. (2010); Fernandes et al. (2017); Daniel (2019); Lawler (1982); Gallie et al. (2012); Rosser & Javinar (2003); Allam & Shaik (2020); Sinha, C. (2012); Mohamed & BedelKhalif (2017); Winter et al. (2000); Efraty et al. (1991); Stein & Kanter (1980); Lai et al. (2012); Rice et al. (1985); Nguyen & Nguyen (2012); Cheung & Tang (2009); Brooks & Anderson (2004); Daniel (2019); Moradi et al. (2014); Tabassum et al. (2011); Laschinger et al. (2001); Pomaki & Maes (2002); Cacioppe & Mock (1984); Goodman et al. (2001); Gillet et al. (2013); Brooks & Anderson (2005); Rosser & Javinar (2003); Alserhan et al. (2021); Tamini & Chadha (2018); Nia & Maleki (2013); Gallie et al. (2012); Edwards et al. (2009); Ngambi (2000); Ahmadi et al. (2011); Sirgy et al. (2008); Siegrist et al. (2007); Kauhanen & Nätti (2015); Ogungbamila & Idemudia (2016); Sawhney & Khatri (2015); Brooks et al. (2007); Rahimiyan & Najafi (2015); Mosadeghrad (2013); Al Kuwaiti & Subbarayalu (2019).

Opportunity to use and develop human capacities – Autonomy, control, performance feedback, skill exploitation and enhancement - Nanjundeswaraswamy and Swamy (2013); Royuela et al. (2008); Da Silva Timossi et al. (2008); Noor and Abdullah (2012); Louis (1998); Tabassum (2012); Shahbazi et al. (2011); Saraji & Dargahi (2006); Royuela et al. (2009); Jabeen et al. (2018); Kulkarni (2013); Normala (2010); Nowrouzi et al. (2016); Dehghan Nayeri et al. (2011); Lee, et al. (2007); Chan et al. (2007); Islam & Siengthai (2009); Cohen et al. (1997); Sirgy et al. (2001); Jorde Bloom (1996); Almalki et al. (2012); Bujang (2010); Koonmee et al. (2010); Fernandes et al. (2017); Daniel (2019); Rosser & Javinar (2003); Allam & Shaik (2020); Winter et al. (2000); Allam & Shaik (2020); Nguyen & Nguyen (2012); Daniel (2019); Tabassum et al. (2011); Yavari et al. (2009); Cacioppe & Mock (1984); Rosser & Javinar (2003); Alserhan et al. (2021); Ahmadi et al. (2011); Kauhanen & Nätti (2015); Ogungbamila & Idemudia (2016); Brooks et al. (2007); Rahimiyan & Najafi (2015); Al Kuwaiti & Subbarayalu (2019).

Opportunity for career growth – Continual growth in career - Nanjundeswaraswamy and Swamy (2013); Beh and Idris (2006); Royuela et al. (2008); Da Silva Timossi et al. (2008); Rossmiller (1992); Lee et al. (2013); Baleghizadeh and Gordani (2012); Noor and Abdullah (2012); Louis (1998); Tabassum (2012); Royuela et al. (2009); Jabeen et al. (2018); Normala (2010); Nowrouzi et al. (2016); Lee, et al. (2007); Chan et al. (2007); Sirgy et al. (2001); Jorde Bloom (1996); Almalki et al. (2012); Bujang (2010); Koonmee et al. (2010); Fernandes et al. (2017); Daniel (2019); Rosser & Javinar (2003); Allam & Shaik (2020); Allam & Shaik (2020); Li & Yeo (2011); Daniel (2019); Tabassum et al. (2011); Cacioppe & Mock (1984); Rosser & Javinar (2003); Alserhan et al. (2021); Siegrist et al. (2007); Kauhanen & Nätti (2015); Brooks et al. (2007); Rahimiyan & Najafi (2015); Mosadeghrad (2013); Al Kuwaiti & Subbarayalu (2019).

Social Integration in the work force – Openness, community, self esteem, upward mobility, unbiased treatment - Nanjundeswaraswamy and Swamy (2013); Royuela et al. (2008); Narehan et al. (2014); Lee et al. (2013); Louis (1998); Tabassum (2012); Bragard et al. (2015); Shahbazi et al. (2011); Adhikari & Gautam (2010); Saraji & Dargahi (2006); Wan & Chan (2013); Mirkamali & Thani (2011); Lee et al. (2015); Normala (2010); Lee, et al. (2007); An et al. (2011); Chan et al. (2007); Vagharseyyedin et al. (2011); Al-Qutop & Harrim (2011); Islam & Siengthai (2009); Fields & Thacker (1992); Hart (1994); Almalki et al. (2012); Eaton et al. (1992); Koonmee et al. (2010); Fernandes et al. (2017); Daniel (2019); Allam & Shaik (2020); Sinha, C. (2012); Allam & Shaik (2020); Daniel (2019); Moradi et al. (2014); Tabassum et al. (2011); Cacioppe & Mock (1984); Alserhan et al. (2021); Ngambi (2000); Sawhney & Khatri (2015); Mosadeghrad (2013).

Constitutionalisation in the work organization – Constitutional protection, fair practices, due process, free speech - Nanjundeswaraswamy and Swamy (2013); Royuela et al. (2008); Rossmiller (1992); Noor and Abdullah (2012); Louis (1998); Tabassum (2012); Bragard et al. (2015); Shahbazi et al. (2011); Dehghan Nayeri et al. (2011); Lee, et al. (2007); Chan et al. (2007); Vagharseyyedin et al. (2011); Al-Qutop & Harrim (2011); Islam & Siengthai (2009); Cohen et al. (1997); Jorde Bloom (1996); Almalki et al. (2012); Fernandes et al. (2017); Daniel (2019); Allam & Shaik (2020); Winter et al. (2000); Efraty et al. (1991); Allam & Shaik (2020); Li & Yeo (2011); Daniel (2019); Tabassum et al. (2011); Cacioppe & Mock (1984); Goodman et al. (2001); Alserhan et al. (2021); Ngambi (2000); Kauhanen & Nätti (2015); Rahimiyan & Najafi (2015); Mosadeghrad (2013).

7.**Work and personal life** – Balancing work and personal life - Nanjundeswaraswamy and Swamy (2013); Elizur and Shye (1990); Arif and Ilyas (2013); Royuela et al. (2008); Narehan et al. (2014); Lee et al. (2013); Noor and Abdullah (2012); Louis (1998); Tabassum (2012); Shahbazi et al. (2011); Saraji & Dargahi (2006); Chan et al. (2007); Cohen et al. (1997); Sirgy et al. (2001); Kraut et al. (1989); Rathi (2009); Almalki et al. (2012); Fernandes et al. (2017); Daniel (2019); Cheung & Tang (2009); Daniel (2019); Tabassum et al. (2011); Cacioppe & Mock (1984); Brooks & Anderson (2005); Alserhan et al. (2021); Tamini & Chadha (2018); Edwards et al. (2009); Sawhney & Khatri (2015); Rahimiyan & Najafi (2015).

Social relevance of work – Emphasis on Social cause - Nanjundeswaraswamy and Swamy (2013); Arif and Ilyas (2013); Royuela et al. (2008); Narehan et al. (2014); Louis (1998); Tabassum (2012); Singhapakdi et al. (2015); Chan et al. (2007); Al-Qutop & Harrim (2011); Sirgy et al. (2001); Jorde Bloom (1996); Almalki et al. (2012); Fernandes et al. (2017); Tabassum et al. (2011); Yavari et al. (2009); Cacioppe & Mock (1984); Alserhan et al. (2021); Rahimiyan & Najafi (2015).

CONCLUSION

Walton (1974) has opined an exhaustive list of dimensions for measuring and most studies conducted after Walton (1974) have utilized these dimensions in studying QWL. It may help managers & budding entrepreneurs to instil and use various dimensions of QWL. It can guide future researchers in finding relationship between Walton (1974) with studies on QWL. A congruence is established between 8 dimensions as given by Walton (1974) and reviewedliterature.

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Table 1. Summary of Studies Reviewed

S. No.	Author(s)	Dimensions studied with Quality of Work Life (QWL)
1.	Bagtasos, M. R. (2011)	Job content and context
2.	Nanjundeswaraswamy and Swamy (2013)	Working environment, culture, Relationship, Training and development; Rewards; Job security and satisfaction,Autonomy, resource Adequacy
3.	Elizur and Shye (1990)	Mode and field of functioning & life areas
4.	Beh and Idris (2006)	Climate, satisfaction, and balance
5.	Arif and Ilyas (2013)	Working Climate, work-life balance, and satisfied relationships
6.	Royuela et al. (2008)	Job quality; continual career development; safe work - places; Flexibility; Inclusive labour market; work-life balance; participative and involvement; non- discrimination; performance
7.	Da Silva Timossi et al.(2008)	Work and satisfaction
8.	Rossmiller (1992)	Performance feedback, development, congruent personaland school goals
9.	Narehan et al. (2014)	Working environment, job aspects, emotional wellbeing, development, inclusion, and inter-personal relationships.
10.	Lee et al. (2013)	Security, workload, work- life balance, staffing
11.	BaleghizadehandGordani (2012)	Working environment, job and motivation
12.	Noor and Abdullah (2012)	Job involvement, satisfaction, and security
13.	Farid et al. (2015)	Commitment
14.	Louis (1998)	Commitment and sense of efficacy
15.	Tabassum (2012)	Compensation, constitutionalism, Development
16.	Bragard et al. (2015)	Working conditions, psychological demands, adequateresources, and support.
17.	Shahbazi et al. (2011)	Development, Constitutionalism, life space and social
		integration, performance

18.	Adhikari & Gautam (2010)	Working environment
19.	Saraji & Dargahi (2006)	Hygiene, Managers, compensation, work - life balance and work satisfaction and development
20.	Wan & Chan (2013)	Job characteristics, policies, relationships, and work environment
21.	Royuela et al. (2009)	Job satisfaction
22.	Mirkamali & Thani (2011)	Integrity, cohesiveness
23.	Jabeen et al. (2018)	Job satisfaction and turnover
24.	Lee et al. (2015)	Health, safety; economic, self-actualization, esteem and social, knowledge and aesthetics
25.	Kulkarni (2013)	Development
26.	Normala (2010)	Development, participation working environment, supervision, compensation, and social recognition
27.	Nowrouzi et al. (2016)	Treatment of new employees, development, relationships, stress-reduction, and compensation
28.	Kanten & Sadullah (2012)	Working conditions
29.	Almalki et al. (2012)	Work satisfaction, turnover, and productivity
30.	Singhapakdi et al. (2015)	Corporate Social Responsibility
31.	Dehghan Nayeri et al. (2011)	Participation and continuous development
32.	Lee, et al. (2007)	Lower and Higher order needs
33.	An et al. (2011)	Organizational culture
34.	Chan et al. (2007)	Esteem, actualization, compensation, family, health, safety, development
35.	Vagharseyyedin et al. (2011)	Leadership style, working environment, compensation, relationships, demographics, and workload
36.	Al-Qutop & Harrim (2011)	Performance, effectiveness, innovation
37.	Islam & Siengthai (2009)	Training, worker union, participation
38.	Cohen et al. (1997)	Self-management leadership
39.	Sirgy et al. (2001)	Need satisfaction and spill-over theories
40.	Kraut et al. (1989)	Computerized record systems and work life
41.	Jorde Bloom (1996)	Innovativeness, goal consensus, career growth, and clarity
42.	Rathi (2009)	Working environment and psychological well-being
43.	Fields & Thacker (1992)	Union and company commitment
44.	Hart (1994)	Psychological distress and morale
45.	Almalki et al. (2012)	Working environment, facilities, work - life balance, staffing, supervision, development
46.	Eaton et al. (1992)	Grievance procedure
47.	Bujang (2010)	Work commitment, stress and satisfaction
48.	Koonmee et al. (2010)	Satisfaction, commitment
49.	Fernandes et al. (2017)	Compensations, working conditions, constitutionalism and life space
50.	Daniel (2019)	Job satisfaction, commitment, rewards, participation, work - life balance, grievance handling, welfare, working environment
51.	Lawler (1982)	Working environment
52.	Gallie et al. (2012)	Intrinsic job quality
53.	Rosser & Javinar (2003)	Job satisfaction and morale

54.	Allam & Shaik (2020)	Autonomy, relationships, recognition, compensation, self-respect, and supervision
55.	Sinha, C. (2012)	Relationship and -sustenance futuristic, professional orientation, self-deterministic and systemic orientation
56.	Mohamed & Bedel Khalif (2017)	Well-being, job satisfaction and working conditions
57.	Winter et al. (2000)	Clarity,motivation, alienation, roleoverload, performance feedback, and decision making
58.	Efraty et al. (1991)	Alienation, job satisfaction and involvement
59.	Stein & Kanter (1980)	Organisation Design
60.	Allam & Shaik (2020)	Autonomy, relationships,recognition, compensation, self-respect, and supervision
61.	Lai et al. (2012)	Role of generation, workload
62.	Li & Yeo (2011)	Career development, autonomy
63.	Rice et al. (1985)	Working environment
64.	Nguyen & Nguyen (2012)	Psychological capital (PsyCap), performance
65.	Cheung & Tang (2009)	Emotional labor, work - family balance
66.	Brooks & Anderson (2004)	Workload
67.	Daniel (2019)	Job satisfaction, commitment, compensation, participation, work - life balance, grievance handling, welfare facilities, working environment
68.	Moradi et al. (2014)	Compensation, personality, accidents, stress, safety regulations and discipline, working conditions, welfare
69.	Tabassum et al. (2011)	Compensation, life space, Development, flexibility, relationships
70.	Laschinger et al. (2001)	Job strain
71.	Pomaki & Maes (2002)	Job satisfaction, burnout, turnover, absenteeism
72.	Yavari et al. (2009)	Development, social relevance
73.	Cacioppe & Mock (1984)	Intrinsic, Extrinsic factors
74.	Goodman et al. (2001)	Organisational culture, commitment, job involvement, empowerment, satisfaction
75.	Gillet et al. (2013)	Distributive, inter-actional justice
76.	Brooks & Anderson (2005)	Work - life balance, shifts, remuneration
77.	Rosser & Javinar (2003)	Job satisfaction and morale
78.	Alserhan et al. (2021)	Happiness
79.	Tamini & Chadha (2018)	Emotional intelligence, career, well-being, work-life
		balance, working conditions
80.	Nia & Maleki (2013)	Commitment
81.	Gallie et al. (2012)	Intrinsic job quality
82.	Edwards et al. (2009)	Satisfaction, well-being, work-life balance,stress, autonomy, working conditions
83.	Ngambi (2000)	Job-sharing
84.	Ahmadi et al. (2011)	Managerial Coaching
85.	Sirgy et al. (2008)	Resources, conflict, role identities and demands, stress
86.	Siegrist et al. (2007)	Autonomy, efforts, rewards
87.	Kauhanen & Nätti (2015)	Development, insecurity, and autonomy
88.	Ogungbamila & Idemudia (2016)	Training, Gender differences

89.	Sawhney & Khatri (2015)	Humanized work, individualization, managerial systems, socio-psychological needs
90.	Brooks et al. (2007)	Job satisfaction, working environment
91.	Rahimiyan & Najafi (2015)	Working environment, decision making, democracy, development, remuneration, medical, welfare
92.	Mosadeghrad (2013)	Compensation, promotion, management support, pride, job security and stress
93.	Al Kuwaiti & Subbarayalu (2019)	Working conditions, psychosocial factors, development, compensation, job satisfaction, security

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RESEARCH ARTICLE

Isolation, Characterization and Identification of Anti – Cariogenic Compounds from the Ethanolic Extract of *Acmella calva* (DC) R.K. Jansen and its Activities

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ABSTRACT

Anticariogenic properties of medicinal plant being used to cure chronic and acute diseases of dental caries, all over the world. In the present investigation, an attempt has been made to isolate and identify the anticariogenic compounds present in the whole plant extract of *Acmella calva* (DC) R.K. Jansen and validate their anticariogenic activities. The bioactive compounds responsible for anticariogenic activity were isolated, characterized and identified by HPTLC, Column chromatography, IR spectrum, FTIR, ^{1}H NMR, ^{13}C NMR, HPLC studies. Screening of anticariogenic activity was carried out with the isolated compounds from the ethanolic extract of whole plant of *A. calva* against four human oral pathogenic bacteria such as *Streptococcus mutans, Streptococcus salivarius, Streptococcus sorbinus and Staphylococcus aureus*. The bioactive compounds Betasitosterol and Quercetin were isolated and identified. Of these two compounds, the maximum zone of inhibition was found in Beta sitoterol against *Streptococcus mutans*, the major causative organism of dental caries. From this study, it is concluded that the selected plant *A. calva*is an efficient source which could be used to cure one of the prevailing disease dental caries of human.

Keywords: *Acmella calva* (DC) R.K. Jansen, dental caries, anticariogenic, pathogenic bacteria.

INTRODUCTION

The use of medicinal plants has been accustomed to treat human disease since prehistorical period. It is not amazing, that interest has grown in plant based natural product to fight infectious disease. According to world health organization (WHO), more than 80% of the world population is dependent on traditional medicine for their primary

health care needs [1]. Extensive screenings of natural products, particularly from plants, for anticaries activity were carried out in several plants. Plants have become a source of potential antimicrobial substances against dental caries [2, 3]. The evaluation for antibacterial agent of plant origin begins with thorough biological evaluation of plant extracts to ensure efficacy, followed by identification of active principles and efficacy of the new drug[4].Thousands of phyto-constituents which have inhibitory effects on all types of microbes. The importance of plant secondary metabolites in medicine, agriculture and industry has led to numerous studies on the synthesis, biosynthesis and biological activity of these substances. Secondary metabolites are chemical produced by plants. These chemicals are not necessary for a cell to live, but it plays a role in the interaction of the cell with the environment. A few secondary metabolites are used as flavours, drugs, dyes, frangrance, insecticide and have great economic value [5]. Polyphenols and phytosterols occupy a unique place in the world as the only bioactive natural product that general in public is aware. The flavonoid and sterols thus play its vital role against wide range of pathogenic microorganisms. Plant sterols or phytosterols are similar to cholesterol. It may help to reduce cholesterol level in humans. Sterols are used to cure heart diseases. Sterols are highly found in vegetable oil and in nuts of many plants. Flavonoids are group of about 4000 naturally occurring polyphenolic compounds, found universally in food of plants origin [6]. Flavonoids are highly responsible for the colour of many fruits, flowers and vegetables. Flavonoids works as anti-inflammatory, antioxidant, anticancer agents [7]. *Acmella calva* (DC) R.K. Jansen. (Local name - Toothache plant) is a rare medicinal herb selected to study the effective active compounds present in it. It belongs to the family Asteraceae. Various parts of the plant is used to manage situations like cardiovascular, wounds, thyroid disorder, paralysis of tongue, throat infection, psoriasis, tincture, dysentery, tumour and used to stimulate secretion of saliva [8, 9]. Hence, the present study was aimed to isolate, characterize and identify the phytocompounds present in the *A. calva* and to screen their anticariogenic activity.

MATERIALS AND METHODS

Collection of plant material

The whole Plant of *A. calva* was collected in the month of October from the Nanjikottai road, Tanjore, Tamil Nadu, India. The fresh plants were washed and shade dried at room temperature for 15 days.

Preparation of ethanol extracts

The ethanol extracts were prepared by soaking plant powder in ethanol using a soxhlet extractor for 10 hr continuously. Using whatman filter paper No. 42 (125mm) the extract was filtered. Under reduced pressure, the filtered extract was concentrated and dried using rotary evaporator. The final condensed dried samples were stored in labeled sterile bottles and kept at -20ºC. The filtrate obtained was used as sample solution for further analysis[10].

HPTLC fingerprinting analysis for steroids and flavonoids

HPTLC finger printing studies were carried out according to the method of Wagner and Baldt. .Different compositions of the mobile phase for HPTLC analysis were tested in order to obtain high resolution and reproducible peaks[11].

Steroids

Sample preparation and TLC: 2µl of test solution and 2µl of standard solution (Solasodine) was loaded as 5mm band length in the 3 x 10 Silica gel 60F_{254}TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase n- Hexane: Ethyl acetate (80:20) up to 77mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV 366nm. Derivatization: The developed plate was sprayed with respective spray reagent Anisaldehyde sulphuric acid reagent and dried at 100°C in hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG

REPROSTAR 3) chamber. Scanning: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 538nm[12].

Flavonoids

Sample preparations and TLC:2µl of test solution and 2µl of standard solution (Kaempferol) was loaded as 5mm band length in the 3 x 10 Silica gel 60F_{254} TLC plate using Hamilton syringe and CAMAG LINOMAT 5 instrument. The samples loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase Hexane: Ethyl acetate: Acetic acid (7:3:0.3)up to 52.2 mm. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was kept in Photo-documentation chamber (CAMAG REPROSTAR 3) and captured the images at Visible light, UV 254nm and UV 366nm. Derivatization: The developed plate was sprayed with respective spray reagent Anisaldehyde sulphuric acid reagent and dried at 100°C in hot air oven. The plate was photo-documented in Visible light and UV 366nm mode using Photo-documentation (CAMAG REPROSTAR 3) chamber. Scanning: After derivatization, the plate was fixed in scanner stage (CAMAG TLC SCANNER 3) and scanning was done at 433nm[13]

Isolation of bioactive compounds by column chromatography

The condensed ethanol extract of whole plantsample was subjected to column chromatography over TLC grade silica gel. The column was first eluted with n-hexane and then increased the amount of n- hexane with ethyl acetate to obtain the fractions[14].

Purifications of bioactive compounds by High performance liquid chromatography (HPLC):

The analytical HPLC system (Shimadzu) was equipped with a diode array detector, a 20µl loop, 200 x 4.6 mm C18 column. Ethanol(HPLC grade, 0.2mm filtered) is used as a mobile phase. The isolated compounds were separated using a mobile phase of ethanol: water (70:30 v/v) at a flow rate of 1.0 ml/min, at 30 °Ccolumn temperature. Injection volume was 40 µl and detection was carried out at 346 nm. The isolated steroid compounds were separated using a mobile phase of acetonitrile and water (90:10 v/v). The isolated flavonoid compounds were separated using a mobile phase of acetonitrile and water (70:30 v/v) [15].

Structural elucidation study of isolated compound

Different spectroscopic methods including UV, FTIR, 1H NMR, 13C NMR and Mass spectrum were used to elucidate the structure of isolated compounds. The UV–visible spectrum of the isolated compounds in ethanol was recorded using a Shimadzu 160A UV–visible spectrophotometer. The Fourier Transform Infrared (FTIR) spectra were recorded with a nominal resolution of 4 cm1 and a wave number range from 400 to 4000 cm^{-1} using the KBr pellet technique. H and 13C NMR spectra were acquired on Bruker WP 200 SY and AM 200 SY instruments (1H, 200.13 MHz; 13C, 50.32 MHz) using TMS as internal standard and CDCL3 as solvent. GC – MS analysis of the compound was performed using Perkin – Elmer GC Clarus 500 system and GC chromatography interfaced to a Mass spectrometry equipped with an Elite – I, fused silica capillary column (30m x .25mm 1D x 1µMdf, composed of 100% Dimethly poly siloxane) [14, 15].

Selection of pathogens

Based on the literature studies, the following dental caries causing microbes were selected, *Streptococcus mutans* (MTCC 890), *Streptococcus salivarius* (MTCC 13429), *Streptococcus sobrinus* (MTCC 33479) and *Staphylococcus aureus* (MTCC 25923). All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India.

Determination of antibacterial activity by disc diffusion method

The disc diffusion method is used to evaluate the antibacterial activity of the each isolated compounds. The isolated compounds (10 mg) were re-dissolved in 1 ml of ethanol, sterilized through Millipore filter (0.22 µm) then loaded over sterile filter paper disc (8 mm in diameter) to obtain final concentration of 10 mg/disc. Ten ml of Mueller-Hilton agar medium was poured into sterile Petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter

paper discs loaded with various concentrations of isolated compounds of 20, 40, 60, 80, 100, 120, 140 and 160 µg/ml and were placed on the top of Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. Negative control was prepared using the ethanol solvent. The plates were incubated at 37 ºC for 24 hours and the zone of inhibition was recorded in millimeter and the experiment was repeated twice [16].

RESULTS AND DISCUSSION

Optimization of the HPTLC chromatographic conditions for the bioactive compounds.

Based on the literature survey the steroids and flavonoids are potent compounds to treat dental caries. Hence the present study was aimed to isolate, identify the steroid and flavonoid compounds present in A. calva and to validate their anticariogenic activity. HPTLC finger print patterns wasevolved for ethanolic extracts of the whole plant of *A. calva* to optimize the steroids and flavonoids present. The standard used for steroid and flavonoid were Solasodine and Kaempferol respectively. Steroid compounds: HPTLC finger prints of *A. calva* were carried by using solvent systemn- Hexane: Ethyl acetate(8: 2) as the mobile phase (Table1;Figure 1) visualized under UV 366 nm before derivation. The ethanolic extract of whole plant of A. calva showed the presence of 5 different types of steroids with different Rf values(0.09, 0.19, 0.24, 0.53, and 0.70). Among the 5 steroids, steroid 3 showed highest peak with Rf value 0.53 and it was used for the identification. Flavonoid compounds: Hexane: Ethyl acetae: Acetic acid [7:3: 0.3] for whole plant, visualized under UV 254 nm before derivatization. The whole plant extract exerted 14 prominent bands with different Rf values (0.01, 0.09, 0.18, 0.30, 0.36, 0.41, 0.46, 0.52, 0.56, 0.9, 0.65, 0.69, 0.77, 0.87, 0.93) Table - 2. Of which only 5 flavonoid compounds were noticed. Among the 5 flavonoids, the 4th flavonoid showed the highest Rf value 0.48 which wasthe peak 7 showed in the Figure - 2 and it was selected for further studies. From this present investigation, it was noted that the steroid compound 3 and flavonoid compound 4 showed the highest peak value. Hence these two phytocompounds were used for further studies.

Isolation of bioactive compounds by Column chromatography

The condensed ethanol extract was eluted with n-hexane and showed 10 fractions; the different combinations of n-hexane with ethyl acetate showed 16 fractions. All the fractions were spotted on a TLC plate until a single spot observed for steroid 3 and flavonoid 4 compounds using the peak value of standard compounds.

Structural Elucidation of isolated compounds

Steroid 3

UV – VIS

The structural elucidation of Steroid 3 was carried out using UV – VIS, showedwaxy white powder with characteristic odor (Rotten egg). The melting point was 136°C. The UV λ_{max} value (The wavelength along the absorption spectrum where a substance has its strongest photon absorption) of compound steroid 3 was 257 nm (Figure 3).

FT - IR

The IR absorption spectrum showed broad absorption peaks at 3437.65 cm^{-1}(O-H stretching) indicating hydrogen bond. The band below 3000cm^{-1} shows aliphatic compound. A long linear chain is identified at 2946.42 cm-1(aliphatic C-Hstretching);the region between 1500-2000^{cm-1} shows double bonded region. The band at 1635.48 cm^{-1}(C=C absorption peak)indicates unsaturated bond. The presence of other absorption band spectra at frequencies 1458.32 cm^{-1}(CH2),1376.47 cm-^{1}(OH def) 1049.23 cm-^{1}(cycloalkane) and 800.64cm^{-1} which sharply indicates hydroxyl group (Figure 4). The compound Steroid 3 is concluded to have carbonyl group in their structure, thus it possesses ketone as their functional group.

^{1}H NMR

In the proton, ^{1}H NMR (CDCl3, 300MHz)has given signals at δ 2.274 (1H, m, H-3), 5.355 (1H, m, H-6), 5.342 (1H, m, H-23), 5.195 (1Hm, H-22), 2.268(1H, m, H-3), 2.274 (1H, m, H-20), 1.816-2.268 (5H, m) ppm. Other peaks are observed at δ 0.763-0.898 (m, 9H), 0.910-1.073 (m, 5H), 1.238-1.477 (m, 4H), 0.697-0.792 (m, 3H), 1.816-2.268 (m, 5H), 1.073-1.123 (m,3H), 1.238-1.543 (m, 9H) ppm (Figure 5). 1H NMR spectrum of Steroid 3, showed one proton multiplet at δ 2.274, the position and multiplicity of which designate 3H of the steroid nucleus. One proton at δ 5.335 showed multiplet as the evident for steroidal skeleton 6H. The spectrum further showed signals at δ 2.268 and δ 0.697 – 0.792 (3H each) designed with two tertiary methyl group. On the other hand triplet of three protons appeared at δ 1.073-1.123. This compound is having six methyl, eleven methylene and three quaternary carbons with hydroxyl group.

^{13}CNMR ($CDCl_3$, 100MHz)

^{13}C NMR has given signal at 37.28 (C-1), 31.90 (C-2), 77.45 (C-3), 42.23(C-4), 140.75 (C-5), 121.74 (C-6), 32.43 (C-7), 31.90 (C-8), 50.12 (C-9), 36.52(C-10), 21.24(C-11),39.68(C-12),42.24(C-13),56.88(C-14),26.08(C-15),28.27(C-16),56.08 (C-17), 36.16 (C-18), 19.44 (C-19), 33.96 (C-20), 26.08 (C-21), 45.86 (C-22), 23.08 (C-23), 12.29 (C-24), 29.15 (C-25), 19.86 (C-26), 19.44 (C-27), 19.04 (C-28), 11.98 (C-29) (Figure 6). Thus, the typical chemical shift at 24 and 29 carbon represent RCH3 group, at the position 19, 23,26, 27 and 28 indicate the presence of R2CH2 group. Carbon at 11, 15, 16, 21 and 25 represent CH3CO group. Position of carbon at 2, 7,8, 10, 18 and 20 shows the presence of R3CH group. The carbon position at 1, 4, 12, 13, 21 and 22 indicate the presence of RCH2Cl and RCH2NH2 group. Position of carbon above 50 that is carbon (3, 9, 14, 17) indicate the presence of RCH2OH group. The carbon position at 5 and 6 represent alkenes (C=C) group. The UV – VIS, FTIR, H NMR and C NMR spectra of the present study compared with NIST library and Spectral Data Base system (Patra*et al*., 2014, Patel*et* al., 2009) [17,18]. The UV λ_{max} showed 257nm. The functional group analysis (FTIR) found the nature of compound as hydroxyl group, which shows positive result for steroid compound. It was found that, the steroid 3 compound was identified as Beta – sitosterol.

Flavonoid 4

UV – VIS

Flavonoid 4 is a yellow precipitate, crystalline in nature and has a melting point 314-315°C and thesubstance showed wavelength at 280 and 375 nm of photon absorption (Figure 7).

FT - IR

The absorption band at3429.34(O-H stretching vibration of phenol) indicates hydrogen bond. A triple bond (C= C) is identified at 2156.40 cm^{-1}(C=O Aryl kenoticstretch).The band range at 1712.86(C---C Aromatic ring stretch)describes simple carbonyl compounds.

The band at 1639.34 (C=O aromatic stretch)mark for unsaturated double or triple bond. The peak at 1568.82(C=C aromatic stretch), 1417.79(O-H bending of phenols), 1367.85(C-H bond in Aromatic hydrocarbon), 1227.82(C-H bond in Aromatic hydrocarbon), 1095.47(C-O stretch of phenol), 1022.53(C-O stretch of Aryl ether), 926.53 and 655.74(C-H bending of aromatic hydrocarbons) sharply indicate hydroxyl compounds(Figure 8). Thus, the compound contains -CHO structure in their functional group. The compound is concluded as aldehyde in nature.

^{1}H NMR

^{1}H-NMR(DMSO-*d*6,500MHz)δ(ppm),8.427(1H,s,OH-3),12.075(1H,s,OH-5),6.295 (1H, d, *J* = 2.0 Hz, H-6), 10.096 (1H, s, OH-7), 8.427 (1H, s, OH-4'), 8.246 (1H, s, OH-3'),7.506 (1H, dd, *J*=2.0, 8.0 Hz, H-6'), 7.672 (1H, d, *J* = 2.0 Hz, H-2'), 6.431 (1H, d, *J* = 2.0 Hz, H-8), 6.956 (1H, d, *J* = 8.5 Hz, H-5') (Figure 9). The 1H-NMR spectra shows a single peak at δ 8.427 ppm indicating carboxylic proton and the signal at δ 12.075 ppm can be assigned to aliphatic proton. The doublet signal at δ 6.295, δ 7.672, δ6.956 and δ 6.431 ppm indicating the aromatic proton respectively. In the same

way the double of doublet signal appeared at δ 7.505 ppm (*J* =2.0) which could be assigned to aromatic proton. One proton of singlet appeared at the signals δ 10.096, δ 8.427 and δ 8.246.

^{13}C NMR

^{13}C-NMR(DMSO-*d*6,125MHz)δ(ppm),148.20(C-2),136.17(C-3),176.35(C-4),161.17 (C-5), 98.67 (C-6), 164.42 (C-7), 93.86 (C-8), 156.58 (C-9), 103.48 (C-10), 120.52(C-1'), 115.53(C-2'),145.50(C-3'),148.20(C-4'),116.10(C-5'),120.52(C-6') Figure 10. Thus C NMR results show that the carbon positions at 6, 8 and 10 indicate RCH20H group. At 1′, 2′, 5′ and 6′ it indicate C=C in alkenes. Carbon position at 2, 3, 3′ and 4′ it describes aromatic rings and at position 4, 5, 7 and 9 it refers to acid and esters (C=0). The observed values for flavonoid 4 was compared with NIST web book and Spectral Data Base system and it is inferred that, the flavonoid 4 shows datas similarity with Quercetin (Selvaraj *et al*., 2013; Thiyagarajan *et al*., 2016) [19, 20]. Thus, the compound flavonoid 4 was identified as Quercetin. The presented study clearly revealed the presence of the bioactive compounds Beta – sitosterol and Quercetinin the ethanolic extract of whole plant of *Acmella calva* (DC.) R. K. Jansenfor the first time. These two compounds were tested for its anticariogenic activities.

Anticariogenic studies of the isolated compounds

The anticariogenic activity of the isolated phytocompounds Beta sitosterol and quercetin of *A. calva* were studied in different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml ,80 µg/ml, 100µg/ml, 120 µg/ml, 140 µg/ml, 160 µg/ml) against dental caries causing pathogens such as, *Streptococcusmutans, Streptococcus salivarius, Streptococcus sorbinus and Staphylococcus aureus*. Ethanol was used as a positive control and Amoxillin was chosen as a negative control. The activity was determined by measuring the 'zone of inhibition' around the disc. The compound beta sitoterol showed antibacterial activity against all four bacteria (*S. mutans, S. salivarius, S. sorbinus and S. aureus*) in the concentration of 160µg/ml, 140µg/ml, 120µg/ml, 100µg/ml, 80µg/ml and 60µg/ml in the maximum range of 11 to 8mm. The lower concentrations (40 - 20µg/ml) showed poor response againstall four bacteria and no zone of inhibition was found in 20µg/ml (Table – 3). The phytocompound quercetin showed the maximum zone of inhibition 9 mm for *S. mutans*, 11 mm for *S. salivarius*, 7 mm for *S.sorbinus*, and 10 mm for *S. aureus* at 160µg/ml. The growth of bacteria highly at 160 - 140 µg/ml concentration. It was followed by 120 and 100 µg/ml, which showed the maximum of 8 mm zone of inhibition against *S. salivarius and S. sorbinus*and showed moderate activity against all other bacteria. The lower concentration (40 and 20µg/ml) showed poor response (Table - 4). The result revealed that *S. mutans*was more sensitive to Betasitosterol followed by *S. salivarius, S. sorbinus*and *S. aureus* than Quercetin. This is because β-Sitosterol is proven to be an effective nutritional supplement, safe, nontoxic and has a very powerful potential health benefits in many diverse applications including antibacterial activity (Sen et al., 2012) [21]. Of the two phytocompounds tested, Betasitosterol (Steroid 3) showed the higher anticariogenic activity against selected dental caries causing microbes. From this study it is concluded that the compound Betasitosterolis a potent bioactive compound to cure the dental caries – a predominant disease affect children's.

CONCLUSION

The HPTLC method was developed for the identification and quantification of Beta - sitosterol and Quercetin from the whole plant of *A. calva.* From the above physical, chemical, spectral evidences, the compounds isolated from ethanolic whole plant extract of *Acmella calva* (DC) R.K. Jansenis confirmed as Beta - sitosterol and Quercetin. The compounds showed good anticariogenic activity against human oral pathogens. Hence, the compounds proved to be very effective against dental caries causing microbes. This is the first ever report of these steroid and flavonoid compounds from this plant.

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Table 1 –HPTLC finger print of steroid compounds present in *Acmella calva* (DC.) R. K. Jansen

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	149.0	0.01	149.0	10.98	0.03	0.0	664.4	1.17	UNKNOWN*
2	0.09	0.4	0.11	25.0	1.84	0.12	23.0	367.7	0.65	STEROID 1
3	0.13	23.5	0.15	28.1	2.07	0.17	9.1	682.2	1.20	UNKNOWN*
4	0.19	4.8	0.21	32.0	2.35	0.23	9.8	575.2	1.01	STEROID 2
5	0.24	10.4	0.25	16.3	1.20	0.27	0.7	244.8	0.43	STEROID 3
6	0.32	0.5	0.43	272.4	20.06	0.53	40.3	13156.0	23.18	UNKNOWN*
7	0.53	40.8	0.59	646.9	47.65	0.65	55.0	33740.1	59.44	STEROID 4
8	0.66	55.1	0.67	62.4	4.60	0.70	44.8	1673.5	2.95	UNKNOWN*
9	0.70	44.6	0.74	76.5	5.63	0.83	0.1	3477.4	6.13	STEROID 5
10	0.84	0.7	0.91	49.0	3.61	0.96	0.2	2184.8	3.85	UNKNOWN*

Table 2–HPTLC finger print of flavonoid compounds present in *Acmella calva* (DC.) R. K. Jansen

Peak	Start Rf	Start Height	Max Rf	Max Height	Height %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	55.9	0.02	485.6	24.93	0.09	70.9	11514.6	21.41	UNKNOWN*
2	0.09	71.0	0.12	127.3	6.53	0.18	44.1	4706.4	8.75	UNKNOWN*
3	0.18	44.3	0.20	79.8	4.09	0.24	0.1	1966.0	3.66	FLAVONOID 1
4	0.30	1.6	0.34	23.5	1.20	0.36	7.6	521.5	0.97	UNKNOWN*
5	0.36	7.9	0.40	20.3	1.04	0.41	19.7	493.4	0.92	FLAVONOID 2
6	0.41	19.3	0.46	179.7	9.22	0.48	155.3	4364.3	8.12	UNKNOWN*
7	0.48	155.6	0.49	170.7	8.76	0.52	97.1	3923.6	7.30	FLAVONOID 3
8	0.52	97.5	0.54	102.6	5.27	0.56	77.9	2602.0	4.84	UNKNOWN*
9	0.56	78.3	0.59	167.8	8.61	0.65	48.1	6525.1	12.14	FLAVAONOID 4
10	0.65	48.2	0.66	53.9	2.77	0.68	38.6	1254.2	2.33	UNKNOWN*
11	0.69	38.8	0.73	229.4	11.78	0.76	44.5	7227.7	13.44	UNKNOWN*
12	0.77	45.3	0.80	106.1	5.44	0.86	0.0	4241.0	7.89	UNKNOWN*
13	0.87	0.4	0.91	113.6	5.83	0.93	22.8	2655.7	4.94	UNKNOWN*
14	0.93	22.8	0.95	88.1	4.52	0.97	54.9	1775.1	3.30	UNKNOWN*

Table – 3 - Anticariogenic activity of the compound beta sitosterol isolated from ethanolic extract of *Acmella calva* (DC.) R. K. Jansen against four dental caries causing organisms

Isolated compounds	Concentration (µg/ml)	Organisms/Zone of inhibition (mm)			
		Streptococcus mutans	*Streptococcus salivarius*	*Streptococcus sobrinus*	*Staphylococcus aureus*
Betasitosterol	20	0.8±0.2	0.7±0.76	0.7±0.2	0.66±0.5
	40	0.3±0.57	0.8±0.34	3.8±0.2	2.06±0.50
	60	4.9±1.01	5.96±0.5	5.6±1.04	4.5±1.80
	80	4.5±1.5	6.08±0.94	7.08±0.3	6.03±0.3
	100	8.4±0.6	6.1±0.9	7.7±0.6	8.1±0.36
	120	6.8±2.02	6.7±0.3	6.3±0.60	8.03±0.20
	140	9.2±1.10	9.03±0.41	8.23±0.50	8.23±0.25
	160	10.2±0.04	8.4±0.75	8.46±0.59	8.73±1.10
	Negative Control (Ethanol)	0	0	0	0
	Standard (Amoxicillin) **Positive control**	8.7±0.86	8.7±0.5	7.66±0.95	8.6±0.55

Values are mean ± standard error of three experiments

Table – 4 - Anticariogenic activity of the compound Quercetin isolated from ethanolic extract of *Acmella calva* (DC.) R. K. Jansen against four dental caries causing organisms

Isolated compounds	Concentration (µg/ml)	Organisms/Zone of inhibition (mm)			
		Streptococcus mutans	*Streptococcus salivarius*	*Streptococcus sobrinus*	*Staphylococcus aureus*
Quercetin	20	3.5±0.5	0.3±0.32	0.23±0.2	0
	40	6.6±0.36	6.3±0.4	5.03±1.3	2.5±0.5
	60	7.5±0.65	6.3±0.81	7.4±0.4	3.5±0.56
	80	8.3±1.7	8.3±0.8	7.76±1.32	5.03±0.45
	100	7.5±1.17	7.5±0.8	8.03±0.25	8.1±0.36
	120	7.2±1.4	8.36±0.63	7.8±0.2	8.23±0.20
	140	7.1±1.22	9.2±0.32	8.03±0.25	8.6±0.45
	160	9.3±0.63	9.4±1.5	8.3±0.73	8.73±1.13
	Negative Control (Ethanol)	0	0	0	0
	Standard (Amoxicillin) **Positive control**	9.13±0.51	8.7±0.3	6.8±0.76	8.2±1.05

Values are mean ± standard error of three experiments

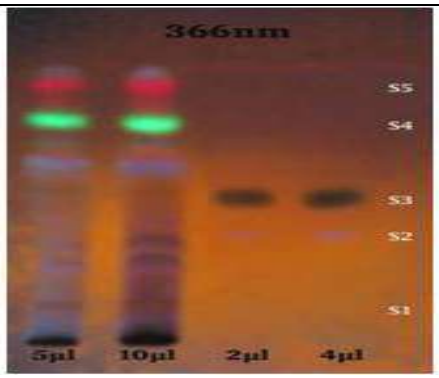

Figure.1 HPTLC band formation of steroid compounds

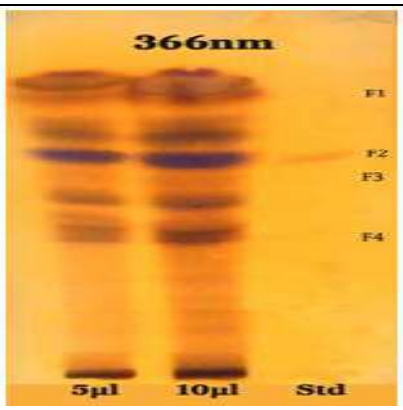

Figure.2 HPTLC band formation of flavonoid compounds

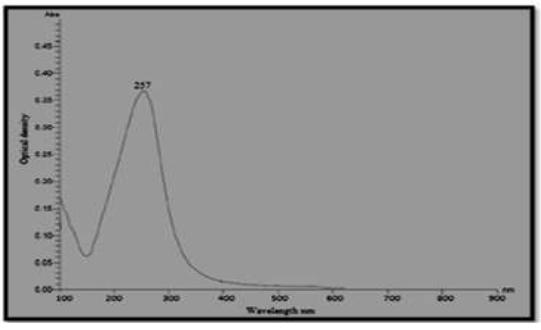

Figure.3 Ultra violet – Visible Spectrum of Steroid 3

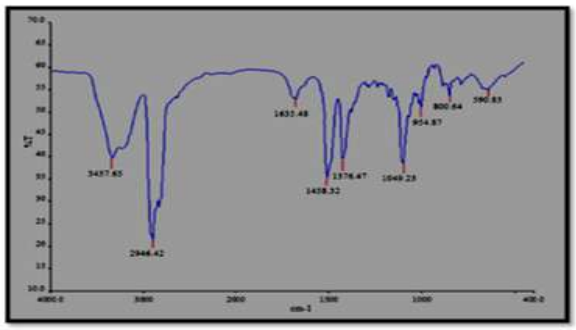

Figure.4 Fourier Transformation – Infra Red of Steroid 3

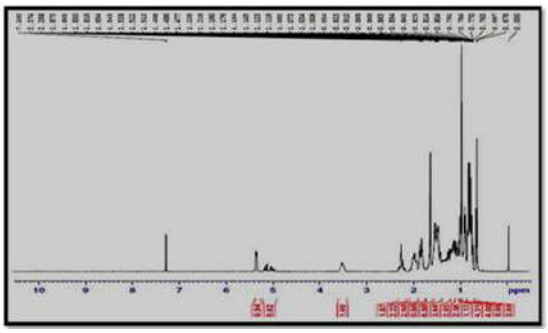

Figure.5 Proton Nuclear Magnetic Resonance (1H NMR) of steroid 3

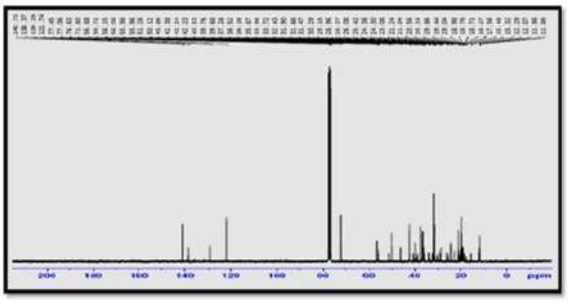

Figure.6 Carbon Nuclear Magnetic Resonance (13C NMR) of steroid 3

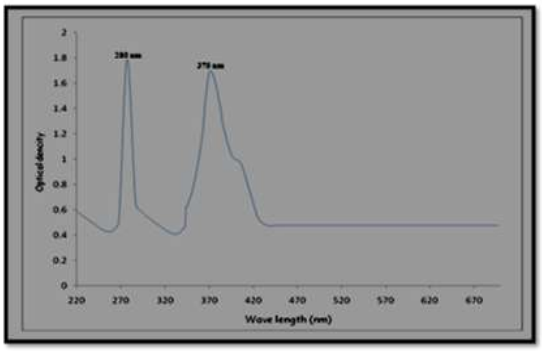

Figure.7 Ultra violet – Visible Spectrum of flavonoid 4

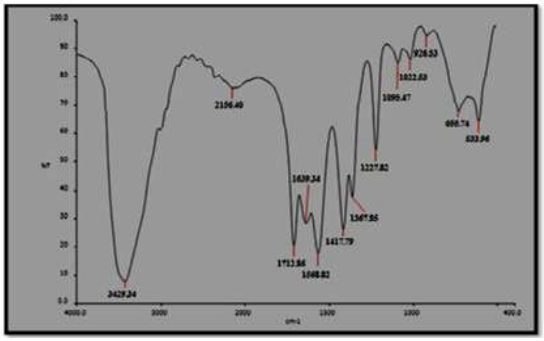

Figure.8 Fourier Transformation – Infra Red of flavonoid 4

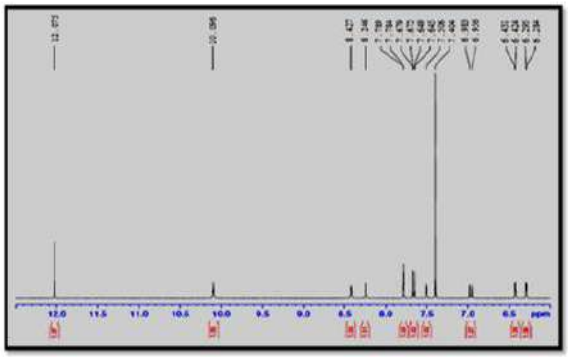

Figure.9 Proton Nuclear Magnetic Resonance (1H NMR) of flavonoid 4

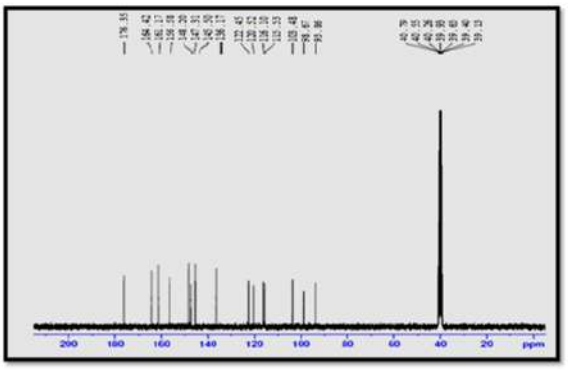

Figure.10 Carbon Nuclear Magnetic Resonance (13C NMR) of flavonoid 4

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RESEARCH ARTICLE

Constraints Perceived in Functioning of Farmer Producer Organisations (FPOs) by the Member Farmers of Odisha State of India

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ABSTRACT

With the mandates of conglomeration of small and marginal farmers in the development of the concept of farmer producer organization (FPO) for providing remunerative price to farmers for their produce, the farmers are still unaware or face several constraints in getting proper benefits of FPOs. This study was conducted with a sample size of randomly selected 131 member respondents from three FPOs in the Cuttack district of Odisha of India by using survey method for collection of data in a three-point scale in an intention to find out different constraints faced by the FPO member farmers. The study found that 68.70 percentage of member respondents strongly agreed on the major constraint as traditional environment of business activities followed by inadequate knowledge base of resource institutions (35.88%). Out of all the marketing constraints, exploitation of middlemen found with higher mean score of 2.687 followed by price fluctuation (2.672) and distress sale (2.595). The member respondents perceived major marketing constraints followed by technical constraints.The complex procedure and collateral issues in loan access has pushed the farmers for external loans with high interest.

Keywords: Constraint,Farmer, Market, Middlemen, Producer

INTRODUCTION

Around 51% of India's geographical area is already under cultivation, but more than 85 percent are of less than 2 ha. of land holdings i.e. small and marginal farmers.Also, agriculture is associated with uncertainties in monsoon and distress sale with markets uncertainty making it one of the risky professions to consider for the youth to adopt their parental occupation. That's why for Agriculture, there are few takers among rural youth by choice. So, effort must seek to attract them towards agriculture by converting the farming into agrienterprise and focusing on small and marginal farmers for better income. Jena and Kanungo (2021) found from their study that factors like education, land holding, average annual income and cosopoliteness, had promoted the intergenerational occupation mobility in farming sector.Umesh et. al. (2019) found that achievement motivation, economic motivation and innovativeness were found to be significantly correlated with the aspiration of the rural youth. In the time of liberalization and privatization of Indian agriculture, the small farmer found himself and his livelihood threatened in an environment of instability, competition and fragmentation of farm holdings. But rather than a lone farmer struggling with multiple circumstances beyond his control, could he not become part of a collective for mutual support and collective action.This has led to policy makers to think of the concept of Farmer Producer Organization (FPO) as aggregation like farmer cooperatives, farmers clubs, farmer interest groups, etc., registered under the Indian Companies Act, 1956. Producer Organizations therefore are supposed to be non-political entities aimed at providing business services to smallholder farmer members, founded on the principal of self-reliance (Onumah*et al*., 2007).

The basic purpose envisioned for the FPOs is to collectivize small farmers for backward linkage for inputs like seeds, fertilizers, credit, insurance, knowledge and extension services; and forward linkages such as collective marketing, processing, and market-led agriculture production (Mondal, 2010). But at the same time, the member farmers are facing severalconstraints which retards the success of FPOs. Chopade*et al.* (2019) revealed in his study that 30.71 percent of the members of Farmer Producer Organization are not familiar with the concept of bank. Evengy and Thomas (2016) concluded that members are confronted with several limitations caused by insufficient capital endowment, weak information channels inside the organization, problems with local leadership and low member contribution. With the mandates of conglomeration of small and marginal farmers, the farmers are still unaware or face several constraints in getting proper benefits ofFPO. Dhakal (2013) revealed that farmers organization's were collapsed due to lack of ownership, group management skill and inability to link with the market. Here, the study was intended to find out various constraints like social, organizational, technological, marketing, and economic constraints perceived by the member farmers of the FPOs whichretardsthe farmers in getting proper benefits of conglomeration.

MATERIALS AND METHODS

The research study was undertaken purposively in the Cuttack district of Odisha state with a perceptual analysis of members of farmer producer organisations (FPOs) to find out its perceived constraints. There are nine FPOs in Cuttack district, out of which three FPOs are active and selected for the study. The total sample size was of 131 member respondents who were selected randomly with 10 percent of total population and the data were collected in 3-point scale (fully agree-3, agree-2, disagree-1) for each statement. The study followed the ex post facto design by accessing the perceived constraints. Survey research through a structured interview schedule was considered most appropriate to gather information. The statistical methods like mean score, ranking and correlation analysis were used to find out the perceived major constraints dominating on others which act as hinderance in the development of the member farmers.

RESULTS AND DISCUSSION

In analysis of constraints perceived by the member farmers with respect to social factors in promotion and function of FPOs (Table 1), there were about 68.70 percentage of member respondents strongly agreed on the major constraint as traditional environment of business activities followed by inadequate knowledge base of resource institutions (35.88%). Majority of the respondents i.e 89.31 percentage disagreed on the criteria of untapping social capital/ community resources followed by divergent interests of the members (51.15%), presence of group conflicts (47.33%) and non-representation of all sections of the area (46.56%). This indicated that FPO has emphasized equal opportunities to all the sections of the farmers with harnessing the local community resources to minimize the cost of cultivation. Absences of group conflicts and political interference help in the successful functioning and sustainability of the FPO. Traditional business activities found with higher mean value of 2.67 and considered as the major social constraints followed by inadequate knowledge base of resource institutions (2.27) and unsupportive policies (2.24) for sustainability and future of FPO.The analysis of organizational constraints (table 2) showed that the FPO members fully agreed on the aspects oflack of decision making (51.91 %), unequal work delegation (45.04%) and weak economic status to run the organization (44.27%) while 96.18 percentage respondents agreed on absence of better forward and backward linkage followed by inability of professionals and staffs (58.02%) to run the FPO. 87.02 percentage respondents disagreed on lack of training in group formation and 68.70 percentage on inadequate managerial capacity and mismanagement of accounts (61.07%). This showed better internal equilibrium of FPO. The FPO promoter agency has facilitated effective training to the members in groupformation. With reference to the mean score results, it was found that absence of forward and backward integration (mean score= 2.96), lack of decision making (2.48) and unequal work delegation (2.42) were the major organizational constraints faced by the FPO members.The technical constraints in functioning of FPOs (Table 3)found maximum farmers(96.18%) fully agreed on prevalence of insufficient storage facilities followed by lack of awareness on importance of grading and packaging (77.86%) and computer Illiteracy (74.05%) while 66.36 and 55.73 percentage member respondents agreed on the constraints, insufficient training and services and difficulties in following recommended practices respectively. The mean score ranking indicated insufficient storage facilities having mean score 2.95 as the major technical constraints perceived by member respondents followed by lack of awareness on importance of grading and packaging and computer Illiteracy with mean score of 2.74 and 2.679 respectively. Insufficient storage facilities and lack of awareness on importance of grading and packaging made the farmers to sell their produces at consumers' price. It has minimized the bargaining power of the producers urging a lower price for their produces.

The computer illiteracy played the hinderance in accessing important information related to their problems, innovation technologies and modern implements from huge internet sources. Improper identification of needs lead to dissatisfaction of the people with the technologies which ultimately leaded to lower adoption of the new technologies and lower the effectiveness of the training programmes. With respect to marketing constraints associated with the FPOs (Table 4), about 70.23 percentage member farmers felt that price fluctuation and exploitation of middlemen were the major marketing constraints followed by distress sale (69.47%) while 61.07 percentage respondents agreed with delayed payments which had retarded them from investment in their next crop. Out of all the marketing constraints, exploitation of middlemen found with higher mean score of 2.687 followed by price fluctuation (2.672) and distress sale (2.595).Exploitation of middlemen was still a hinderance for higher share of farmers in consumers' price. It might be due to illiteracy and unawareness of the farmers on marketing and mandi system or lack of contact of potential buyers.The analysis of economic constraints (Table 5) found 65.65 percentage member farmers agreed fully on statements like unaware of credit facilities and lack of crop insurance facilities followed by high-costlabour (61.83%). It was found that high cost of labour (mean score= 2.55) followed by unaware of credit facilities (2.511) were the major economic constraints perceived by the FPO member respondents. High cost of labour had hiked the cost of cultivation and collateral securities problem in getting bank credit facilities had retarded the farmers for higher investment in cultivation for improved varieties and recommended fertilizer application. The table 6 indicated that the member respondents perceived major marketing constraints (mean score= 2.55) followed by technical constraints (2.418) and economic constraints (2.35). Due to major marketing constraints,

the farmers were still far away in getting remunerative prices for their produce which might help them to invest more for better production and motivate them to adopt new technologies for better agriculture. The correlational analysis (Table 7) showed that the independent variables like caste, family size, farming experience and average land holding size showed statistically significant correlation with the social constraint while caste, annual average income and family size have significantly correlated with the organisational constraint. The independent variable caste has showed significant correlation relationship with technical constraints. The variables like age, extension contact and social participation were found to be significantly correlated with dependent variable marketing constraints whereas variable caste had positive statistically significant relationship with economic constraint and negative relationship exist with respectto independent variable extension contact. With more of extension contact and social participation of the member farmers lead to decrease in marketing constraint by facilitating better marketing opportunities.

CONCLUSION

The members of FPOs have harnessed the opportunities in conglomeration of several farmers in reducing their input and transport cost and capacity building on innovation technologies for better production. The more benefits were obstructed by some of the technical and economical constraints. The complex procedure and collateral issues in loan access has pushed the farmers for external loans with high interest. Still the farmers are facing marketing issues in getting remunerative prices for their products due to exploitation of the middlemen and lack of awareness of the farmers regrading market availability and demand. There is a need for a greater recognition of the importance of linking with other actors who are potential sources of services, information, technical support and market outlets with strengthening backward and forward linkages. More importantly, from the supply side, strengthening the capacity of service providers and external actors (government, NGO, church-based, and private sector) will be needed to complement strategies supporting linkages. State governments may encourage FPOs to perform as agencies for procurement operations and extend to develop forward linkages through storage, postharvest processing, value addition for enhanced access to fair market.

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Table1. Social Constraints Perceived by Member Farmers

Sl. No.	Statement	Disagree (%)	Agree (%)	Fully agree (%)	Mean Score	Ranking
01	Awareness and limited interest in FPO	32.82	46.56	20.62	1.878	VI
02	Low literacy rate	11.45	54.96	33.59	2.221	IV
03	Group conflicts	47.33	38.93	13.74	1.664	IX
04	Inadequate knowledge base of resource institutions	9.16	54.96	35.88	2.267	II
05	Divergent interests	51.15	32.06	16.79	1.656	X
06	Non—representation of all section of the area	46.56	29.01	24.43	1.779	VIII
07	Traditional business activities	1.53	29.77	68.70	2.672	I
08	Political affiliation of members	43.51	27.48	29.01	1.855	VII
09	Mixed & conflicting objectives	11.45	59.54	29.01	2.176	V
10	Unsupportive policies	6.87	61.83	31.30	2.244	III

Table2. Organizational Constraints Perceived by Member Farmers

Sl. No.	Statement	Disagree (%)	Agree (%)	Fully agree (%)	Mean Score	Ranking
01	Lack of cooperation and team work among group members	58.78	33.59	7.63	1.489	X
02	Lack of training in group formation	87.02	9.92	3.06	1.160	XII
03	Unequal work delegation	3.05	51.91	45.04	2.420	III
04	Lack of decision making	0	48.09	51.91	2.481	II
05	Absence of forward and backward integration	0	96.18	3.82	2.962	I
06	Weak economic status to run the organization	0.77	54.96	44.27	2.435	IV
07	Inadequate managerial capacity	68.70	20.61	10.69	1.420	XI
08	Mismanagement of accounts	61.07	27.48	11.45	1.504	IX
09	Inefficient monitoring	57.25	28.24	14.51	1.57	VIII
10	Inability of staffs	15.26	58.02	26.72	2.115	VI
11	Political influence	45.80	29.01	25.19	1.794	VII
12	Few executive members handle all responsibilities	6.87	55.73	37.40	2.305	V

Table 3. Technical Constraints Perceived by Member Farmers

Sl. No.	Statement	Disagree (%)	Agree (%)	Fully agree (%)	Mean Score	Ranking
01	Poor quality Input	35.88	21.37	41.98	2.069	VIII
02	Lack of proper infrastructure (Implements, Irrigation facilities, power, and electricity)	4.58	45.80	48.85	2.450	IV
03	Difficulties in following recommended practices	4.58	55.73	38.93	2.344	VI
04	Improper identification of needs	11.45	40.46	47.33	2.359	V
05	Computer Illiteracy - unable to derive	7.63	16.79	74.05	2.679	III

	benefits of ICT					
06	Lack of awareness on importance of grading and packaging	5.34	15.27	77.86	2.740	II
07	Insufficient training and services	2.29	63.36	34.35	2.321	VII
08	Insufficient storage facilities	0.76	3.06	96.18	2.95	I

Table 4. Marketing Constraints Perceived by Member Farmers

Sl. No.	Statement	Disagree (%)	Agree (%)	Fully agree (%)	Mean Score	Ranking
01	Price fluctuation	3.82	24.43	70.23	2.672	II
02	Lack of latest market information	23.66	58.78	17.56	1.939	IX
03	Low price for produce and distress sale	11.45	17.56	69.47	2.595	III
04	Sudden increase in local prices	12.98	38.93	47.33	2.344	VI
05	Coordination problem from production to consumption	15.27	42.75	41.22	2.267	VIII
06	Distant market and high cost of transportation	6.11	45.80	47.33	2.420	V
07	Exploitation of middleman	3.05	25.19	70.23	2.687	I
08	Perishable nature of products	12.21	19.85	66.41	2.557	IV
09	Delayed payments	4.58	61.07	33.59	2.298	VII

Table5. Economic Constraints Perceived By Member Farmers

Sl. No.	Statement	Disagree (%)	Agree (%)	Fully agree (%)	Mean Score	Ranking
01	High cost of labour	6.87	30.53	61.83	2.550	I
02	Lack of sufficient finance	7.63	39.69	51.15	2.450	IV
03	Unaware of credit facilities	16.03	16.79	65.65	2.511	II
04	Lack of crop insurance facilities	16.79	16.03	65.65	2.504	III
05	Difficulty in external loans	6.11	43.51	49.62	2.443	V
06	Lack of adequate accounting system	30.53	50.38	18.32	1.878	VII
07	High interest burden from financial institutes	29.77	29.01	40.46	2.115	VI

Table 6. Overall Constraint Mean Score and Ranking

Sl. No.	Constraints	Mean score	Ranking
1	Mean Social Constraints	1.962	V
2	Mean Organizational Constraints	1.971	IV
3	Mean Technical Constraints	2.418	II
4	Mean Marketing Constraints	2.550	I
5	Mean Economic Constraints	2.350	III

Table7.correlation of independent variables vs. Constraintsof FPO.

Variable Names	Social Constraint	Organizational Constraint	Technical Constraint	Marketing Constraint	Economic Constraint
Age	0.02	-0.06	-0.11	0.28***	0.1
Caste	0.31***	0.19**	-0.23***	0.03	0.16*
Education	0.05	0.05	-0.04	0.08	0.14

Annual Income	0.09	0.15*	-0.02	0.04	-0.03
Family Size	-0.15**	-0.15*	0.11	0	0.02
Farming Experience	0.18**	0.14	-0.14	-0.12	-0.05
House type	0.05	0.14	0.03	0.11	0.18**
Holding Size	-0.2**	-0.07	0.13	0.09	0.05
Family Type	0.01	-0.02	0.09	0.05	0.11
Extension Contact	0.11	-0.09	-0.05	-0.16**	-0.19**
Social Participation	0.14	-0.03	-0.08	-0.16**	-0.06
Source of Information	0.12	-0.02	-0.04	0.04	0.04

Note: *,** and *** represents significance at 10, 5 and 1 % levels.

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REVIEW ARTICLE

Pharmacotherapeutic Management of Pain in Dentistry – A Narrative Review

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ABSTRACT

Pain is one of the most common reasons patients seek dental treatment. It may be due to many different diseases/conditions or it may occur after treatment. Managing acute postoperative pain is fundamental to every dental practice. Various analgesics are available, and the recent introduction of new agents provides even more options from which to choose. To formulate regimens properly, it is essential to appreciate basic pharmacological principles and appropriate dosage strategies for each of the available analgesic classes. Although a number of pharmacological drugs are available in the market, a significant percentage of the population in India prefers alternative herbal medication for relief from dental pain due to the side effects and interactions of pharmacological treatment. The purpose of this article is to provide a brief review of the drugs that should be considered for the management of acute postoperative dental pain. This article also gives idea about different modalities for management of dental pain other than analgesics.

Keywords: Pain, Pharmacotherapy, NSAIDs, Opioids, Acute Dental Pain, Tramadol.

INTRODUCTION

Pain could be a complicated event consisting of a specific sensation and therefore the reactions elicited by that sensation. The dentist's task is to preserve and restore patient's health and to reduce their suffering. Pain is defined as "an unpleasant sensory and emotional experience related to actual or potential tissue injury, or represented in terms of such damage" [1]. Monheim has defined it as: "An unpleasant emotional experience usually initiated by noxious stimulus and transmitted over a specialized neural network to the central nervous system where it is interpreted as such"[2]. First and foremost step in treating of any condition is precise and correct identification of the condition, what's reason for pain, concerned anatomy of the area and condition accounting for it. Pain starts as a result of modulating conditions of excitory and restrictive mechanism of physical and emotional reactions that occur in creature as distinct people. Presentation of pain is particular to particular patient. Here the role of trained dental practitioner is a lot of as a healer than as a specialist. Dentists practiced to treat such patients and patients were able to settle for the treatment offered to them [3].

Classification of Pain
Etiopathogenic classification of pain[4]
Pain due to localised causes
1.Pathologic changes in teeth and jaws
2.TMJ and associated muscles of mastication
3.Nasal and Para nasal diseases
4.Oral mucosal diseases
5.Lymphoid tissue diseases
6.Salivary gland diseases
7.Diseases of blood vessels

Pain close to nerve trunk and central pathways
1.Trigeminal neuralgia (tic douloureux) and glassophyrangial neuralgia
2.A typical facial palsy
3.Migraine and other types of headache

Referred pain from different organs
1.Orophyrangial diseases
2.Diseases of ENT
3.Cervical spondalities
4.Angina pectoris

Temporal Classification
Acute pain
No IASP definition for acute pain, that has been defined as, "pain of recent onset and probable limited duration; it usually has an identifiable temporal and causal relationship to injury or disease" [5].

Chronic (persistent) pain
There's no IASP definition of 'chronic pain' yet, it's normally defined as, "pain lasting greater than 3 or 6 months duration[6] or pain that persists past the normal time of (tissue) healing [7]. Exceptions for second part of definition are chronic inflammatory arthropathy (rheumatoid arthritis), neuropathic pain or hyperalgesia.

Neurobiology and Pathophysiology of Dental Pain
Noxious pain stimuli like microorganism infections, chemical or mechanical erosion of enamel, and gingival recession are foremost routine causes of dental pain. Patent dentinal tubules are the primary structures causing

dentinal pain signal transduction, once the dental insult is in. In keeping with hydrodynamic theory, movement of fluid among the dentinal tubules induces pain via pain fibers around the odontoblastic processes and at the pulp dentine junction.[8,9]The other structure concerned in pain transmission may be a dense network of trigeminal sensory axons in close proximity to odontoblasts. The nerve supply of the dentin odontoblast pulp complex is especially prepared of mechanosensitive nociceptors, viz, A fibers (both δ and β) and C fibers [Figure 1] that by selectively expresses TRPV 1 receptors. The A fibers are in charge of localisation and transmission of quick, sharp pain directly through neural structure. The C fibers get influenced by several modulating interneurons before reaching the neural structure, therefore leading to a slow pain that is usually characterised as dull and aching. Usually C fibers are accountable for referred pain. It is seen that excitement of A δ fibers appears to have a negligible impact, whereas activation of C fibers will rise pulpal blood flow. This increase in pulpal flow is induced by C fibers by neurokinins, particularly substance P (SP), which is liberated from C fibers nerve terminals and is found in inflammation and in pain both.[10] Neurogenic inflammation because of peripheral release of neuropeptides causes changes in vascular permeability of the pulp.[11] Through the A and C nociceptors, pain (action potential) reaches to the dorsal horn of the medulla spinal is. From dorsal horns, pain signals travel to thalamus via the spinothalamic tract. Thalamus acts as a relay station for processing the pain. Pain signals are then transmitted to somatosensory cortex to localize and characterize the pain. Cortex sends signals to descending pathway to modulate (change or inhibit) the pain impulse. These descending fibers release substances (endogenous opioids, serotonin, and norepinephrine) that bind to the opioid receptors and prevent the release of the neurotransmitters such as glutamate or SP, thereby obstructing the pain signal from being transmitted.

Management of Acute Dental Pain

Three- dimensional approach

The "3-D" principle is employed in the following order to manage pain in day to day dental practice

1. Diagnosis
2. Dental treatment
3. Drugs.

METHODS OF PAIN MANAGEMENT

Removing the root cause

It is imperative that any removal leaves no permanent environmental changes in tissue since this condition would be able to create the impulses even though the causative factor had been removed. This method clearly affects pain perceptions.[12]

Obstructing the pathways of painful impulses

This is often the most universally used technique throughout minor oral surgeries in dentistry. During this technique the acceptable local anesthetic is injected in tissue proximity to the nerve concerned. The local an aesthetic solution deters depolarisation of nerve fibers at the area of absorption that ultimately prevent fibers from conducting any impulses centrally beyond that point. The block will be effective as long as the solution is present in sufficient concentration in nerve proximity to prevent depolarisation

Pharmacotherapeutic management of pain

This is commonly a pillar in the treatment of dental pain. Analgesics are frequently utilized in the treatment of any type of pain. Other categories of pharmacotherapeutics are also used for palliative and cause related therapy. Some agents bind to recognised receptor, some agents (agonists) mimic closely to the action of natural substance and a few prevents these actions like antagonists or blocking agents. E.g., Analgesic like, morphine(agonist) bind to mu and kappa receptors that commonly react with natural endorphins (ligands). Naloxane acts as a medication in hindrance of this interaction Aspirin has anti-inflammatory action by cyclo-oxygenase enzyme. Cyclo-oxygenase metabolizes arachidonic acid to prostaglandins whenever there's local tissue injury. All the medicines have desirable and undesirable effects both. Whenever combined drug therapy is the need of an hour then their interaction ought to be understood fully. Some medications act as synergist to the action of others. One should be aware of drug's half-life and its plasma concentration for proper regimen.

Numerous things to be known for concerning medications are: indications, contraindications, incompatibility mode of action, mode of administration, safety, toxicity, complications, idiosyncrasy, anaphylaxis, hypersensitivity reaction and other undesirable reactions [13].

Analgesic Agents

Generally the target of analgesic shouldn't be to eliminate pain solely. Pain has some importance in observing progress in patient's condition. It assists as well as guides the patients once its actions are excessive or abusive. The chief objective of analgesic is comfort the patient to bear the pain [14].

Types

Non-narcotic analgesics
Narcotic analgesics
Adjuvant analgesics

Non-narcotic analgesics

These comprise aspirin and NSAIDS which have analgesic, anti-pyretic, anti-platelet, anti-inflammatory actions. These agents prevent the formation of prostaglandin E1 by inhibiting cyclooxygenase enzyme. The prime advantage of these drugs is that they don't develop tolerance, physical dependence or addiction. They have ceiling effect which means that even if the dose in increased beyond the peak point no added effect of analgesia will be there, however could have an effect on length of analgesia. Acetaminophen and chloride magnesium tri-salicylate are different non-narcotic analgesics. They lack anti pletelet and anti inflammatory action. These analgesics are ordinarily utilized in treatment of mild to moderate pain and chronic pain. Aspirin and NSAIDS are contraindicated in patients taking anticoagulant therapy and other coagulation disorders [15].

Effects and contraindications of NSAIDs [16]

Therapeutic effects

- Analgesic
- Anti-inflammatory
- Antipyretic
- Antidysmenorrheal
- Antiplatelet action (ASA only)

Adverse effects

- Dyspepsia
- Gastric mucosal injury
- Increased bleeding
- Possible urinary tract infections
- Anaphylactoid reactions

Contraindications

- Gastric ulcers or gastrointestinal disease
- ASA or different NSAID-induced hypersensitivity
- ASA-induced asthma and nasal polyps
- Bleeding considerations
- Third-trimester of pregnancy
- Significant kidney illness
- Children (for ASA only)

Concurrent use of the subsequent drugs

- Antihypertensives like angiotensin-converting enzyme inhibitors, diuretics or beta-blockers: NSAIDs could also becoprescribeds if needed for 4 days or less
- Lithium
- Anticoagulants (warfarin)
- Antineoplastic doses of immunosuppressive drug
- Alcohol
- Digoxin if patient is old or has kidney illness
- Other NSAIDs or acetaminophen; long term
- Oral hypoglycemics (for ASA only)

General rules for the prescription of analgesics [16]

- Eliminate the origin of pain, if it is likely
- Individualize regimens depend on pain severity and sort of pain and case history
- Maximize the dose of nonopioid before adding an opioid
- Optimize dose and frequency before change

•For NSAIDs, consider:
preoperative dose
loading dose
prescribing round-the-clock instead of prn (when necessary) on first day
•Avoid continuous use of any analgesic whenever possible
•Reduce the dose and continuation of any NSAID or opioid as far as possible

Narcotic analgesic
Includes morphine and morphine like medicine which act by
1. Depressing nociceptive neurons and activate non-nociceptive cells.
2. Raises the threshold for painful stimuli
3. Changes the emotional reaction to pain
4. Induces sleep that conjointly elevates pain threshold

The action of analgesic is by inhibition of release of bradykinin when mediated by neural mechanism. Addition of opiate to NSAIDS elevates analgesic effect. Narcotics are largely helpful in treatment of severe acute pain and a chronic cancer pain however they're contraindicated in chronic orofacial pain. These drugs cause constipation, so it should be supplemented with stool softener and laxatives. They must be administered at regular time interval to induce maximum impact. This increase the analgesic effect of different medications.[17] Practitioner should be observant for

1. Tolerance =larger dose is needed to get satisfactory analgesic effect.
2. Physical dependence= withdrawal symptoms severe the condition
3. Addiction=compulsive caring for the drug and also its use for effects aside from pain relief.

Hydrocodone and Oxycodone
These are more a lot of attention-grabbing analgesics compared to opiate. The parent medicine has higher affinity for opioid receptors than opiate. Hydrocodone is demethylated to oxymorphone in adequate quantities and each the parent drug and its active metabolite have analgesic effect.[18]In distinction, the analgesic impact of oxycodone is sort of entirely attributed to the parent drug as a very less amounts are demethylated to oxymorphone. Even the lower doses of these drugs are enough for analgesic effects thereby reduce the incidence of nausea as compared to codeine. Unfortunately, equal doses for these opiate derivatives were poorly understood previously which aroused combination product containing irrational dosage formulations. 200 mg codeine, 30 mg hydrocodone, and 20 mg oxycodone are equivalent oral doses, and they have as same analgesic effect as standard regimen of morphine 10 mg IM or 30 mg PO. Codeine doses are well studied, and it is found that the dose of codeine is close to twenty times the IM dose of morphine (200mg vs 10 mg) however clinical studies are insufficient and support an equivalent ratio. Beaver *et al* [19] found that oxycodone10 mg was comparable to 100 mg codeine, and this is able to extrapolate to oxycodone 20 mg and codeine 200 mg. Studies by Hopkinson[20] and by Beaver [21] have shown that hydrocodone 10mg was comparable to codeine 60mg, and this is able to conclude to 33mg hydrocodone and 200mg codeine. Commonly several patients give previous history of nausea as an allergic reaction. However, IgE antibodies are detected that react with many opioids, together with codeine.[22] Most of the opioids are competent in triggering degranulation of mast cells that ends up in the direct release of histamine.[23] Whenever patient reports with any clinical sign of hypersensitivity then it is dentist's duty not to give any drugs from the morphine and codeine family (eg, propoxyphene, pentazocine).[24]

Meperidine
Meperidine 75-100 mg and morphine 10 mg after IM administration are equivalent. Part of meperidine is converted to normeperidine that has no analgesic properties. However it is stimulant for CVS and CNS. Normepridine has a half life of 15-20 hours and its parent drug has half life of 3 hours.[25]Oral bioavailability for meperidine is approximately 25% with oral dose of 300mg which is equivalent to its IM dose of 75 mg. This higher oral dose leads

to a fair greater risk for accumulation of normeperidine. Meperidine may be a poor choice for analgesia due to its poor absorption and accumulation.

Propoxyphene

This drug is on the market just for oral administration. The equianalgesic dose compared with morphine has not been proven, however its efficiency is low. 100 mg is found to be equianalgesic to oral codeine dose of 60 mg. Proxyphene is converted to norpropoxyphene, an activated form. This stimulant has a half life of 30 hours. This drug is cautiously used in mild to moderate pain for short duration.

Pentazocine

Pentazocine is the solely oral agonist antagonist analgesic on the market in US. It has twin action. It produces its agonist impact at kappa receptors whereas antagonist impact at mu receptors. Therefore when it's given with traditional mu agonist opioids simulataneously, it reverses their effect. In contrast to mu agonists, which give unlimited analgesic effectivity, kappa agonists exhibit a ceiling to their analgesic effect, and no advantage comes by increasing doses beyond 50 mg. When concomitant opioids are given, pentazocine acts as an opioid antagonist thereby producing analgesic effect. It should be avoided in opioid dependent and risk of withdrawl patients. It is an attractive choice for patients who have a previous history of opioid abuse because it does not provide euphoric effects mediated by conventional mu agonists. As it is not mu receptor agonist problem of constipation is least.

Tramadol

Tramadol is a centrally acting analgesic with dual action. It's not classified as a controlled substance in United States. The parent drug hinders the uptake of norepineprine and serotonin. This mimics the action of tricyclic antidepressant and potentiates descending neural pathways that inhibit incoming sensitive impulses. This action is more practical in chronic pain management. But its use in the management of acute pain is not well justified. The drug interactions of tramadol and codeine are found to be similar. Tramadol is not indicated in patients with opioid abuse and dependence.[26]Tramadol-acetaminophen combination is available but it is not more effective than codeine-acetaminophen combination.[27] Adjuvant analgesics have independent analgesic activity in precise things. These embrace tricyclic antidepressant, antihistamines, caffeine, steroids, phenothizines and anticonvulsants.

Effects and contraindications of opioids

Effects

- Analgesia
- Antitussive
- Sedation
- Nausea
- Vomiting
- Constipation
- Mood alteration (euphoria/dysphoria)
- Respiratory depression
- Tolerance if for longer duration
- Physical dependence if for longer duration
- Addiction potential
- Miosis (except for meperidine)

Contraindications

- Severe chronic respiratory disorder
- Severe inflammatory intestine illness
- Concurrent use of alcohol
- For meperidine only: enzyme oxidase inhibitor use within the past 14 days

Appendix B: American Dental Association Statement on the utilization of Opioidsin the Treatment of Dental Pain

1. Whenever prescribing opioids, dentists should keep in mind the medical and dental history of the patients, look for current medications and its interactions with the analgesics. Any history of drug abuse should also be considered
2. Always keep a watch on Center for Disease Control (CDC) and State Licensing Boards recommendations for safe opioid prescribing.
3. Dentists should register themselves with a prescription drug monitoring program (PDMP) and employ it to promote the acceptable use of controlled substances for legitimate medical functions, whereas deterring the misuse, abuse and diversion of those substances.
4. Dentists should have a discussion with patients concerning their duties for preventing misuse, abuse, storage, and disposal of prescription opioids.
5. Dentists should contemplate treatment choices that utilize best practices to forestall exacerbation of or relapse of opioid misuse.
6. Dentists should contemplate nonsteroidal anti-inflammatory analgesics (NSAIDs) as a first choice of drug for management of acute pain.
7. Dentists should acknowledge multimodal pain methods for the management of acute pain as a method to avoid the use of opioid analgesics.
8. Dentists should always coordinate with alternative treating doctors, together with pain specialists, once prescribing opioids for management of chronic orofacial pain.
9. Dentists who are practicing ethically and using skilled judgment concerning the prescription of opioids for the treatment of pain mustn't be control liable for the willful and deceptive behavior of patients who successfully obtain opioids for non-dental purposes.
10. Dental students, residents and practicing dentists are inspired to hunt continued education in addictive illness and pain management as associated with opioid prescribing.

Anesthetic Agents

They are used for binary purpose like in diagnosing the pain and managing the pain as well. It's offered in topical and in injection form.

Topical

It is available as solution, spray, and ointments. Water-soluble ointment that contains local anaesthetic and is germicidal are used for managing dental alveolitis. Analgesic balms provide soothing palliative relief in the cases of inflammatory pain of superficial deep classes once applied locally to exposed tissue. Aloe verajuice is historical medicine for superficial pain; balsam of pura; eugenol and guaiacolar alternative well-known balms. These are helpful in dominant of pain from exposed/ulcerated tissues and mucogingival tissue, exposed dentin and acute alveolitis.[28,29]

Injectable Topical Anaesthetic (LA)

Varity of LA is in market with totally different concentration with or without vasoconstrictive. Long acting LA like bupivacineHCl, (marecine) are helpful. Although long acting anesthetics have higher risk of toxicity, adequate dosage, technique of administration, adequate precaution, readiness of emergency are essential for safety and effectiveness of all LA. Extreme caution is needed when administering vasopressor agents in patients receiving MAO inhibitor or anti-depressant of triptyline type as this result in severe prolonged hypertension. Hence most unwanted reaction with LA is critical intravenous injection. Repeated injection of 0.02% morphine sulfate around peripheral nerve has been used to yield LA that is equivalent to bupivacine in onset and duration of dose of 1 mg. or less of morphine without affecting systemic health.[30]

Anti-inflammatory Agents

Numerous different non steroidal medications are use for their anti-inflammotory action in addition to analgesic action. They have very low analgesic and antipyretic effect. Their action is by inhibition of prostaglandin's biosynthesis. These drugs actually work by suppressing inflammation without altering the disease. Corticosteroids are potent anti-inflammatory drugs which act by inhibiting prostaglandin synthesis; their suppressive effect on inflammation could mask infection. They are contraindicated in systemic fungal infections like Herpes Simplex infection [3].

Muscle Relaxants

These are commonly used in management of muscle pain. The muscle relaxants have anti cholinergic action like succinylcholine and methocarbamol. Patient can't stay ambulant for extended period of time or can't continue his usual activity, so such medicines are often used for hospitalized patients under observation [31].

Antidepressants

Tricyclic opposing depressant agents increases the avalibiliity of serotonin and non-epinephrine in the CSF. The dimethylated tricyclic antidepressant medicine produces serotonin proportionately more available and induces some sedative effect. The monomethylated tricyclics makes non-epinephrine proportionately more available and induces CNS stimulation. It's been shown that low dose of amitriptyline 10 mg. simply before sleep will have analgesic effects on chronic pain after many weeks of use. All the anti-depressant drugs should be used under strict supervision as there are chances of hypertension crisis. Major tranquilizers like phenothizinesar helpful in pain management by decreasing modulating effect on anxiety and apprehension. Major tranquilizers like meprobromate and diazepam have advantage of less side effects. There muscle relaxation is beneficial however drug tolerance, dependence and addiction are common side effects. If these drugs are used it should be prescribed for restricted period or totally different medicines should be used systematically.[32]

Vocative agents

Neurovascular pain may be influenced by alpha androgenic blocking action of ergotamine tart rate, which causes stimulating effect on smooth muscles of peripheral and cranial vessels. It is available with or without addition of caffeine. Caffeine enhances vaso-constricting effect Vocative agents. This combination is contraindicated in coronary heart diseases, pregnancy and increased blood pressure [33].

Non-epinephrine blockers

Guanethiine and reserpine appears to block the uptake of non epinephrine by sensitized axons used in treatment of orofacial pain by blocking satellite ganglion. These are commonly used in rheumatoid arthritis.[34]

Antimicrobials

These are introduced systemically after culture and sensitivity testing only. The decrease in pain is by decreasing the inflammation caused by microbial enzymes and byproducts [35].

Antiviral agents

Acyclovir and Vidarabine used in HSV1 and HSV2. These are also effect against Varicella Zoster virus that causes herpes zoster.[36]

Antihistamines

Counteract vasodilating action of histamine by blocking histamine receptor is useful in allergic responses in Neurovascular pain [37].

Anticonvulsive agents

Phenyntoin sodium is an anticonvulsant agent which is capable of suppressing pain in about 20% of paroxysmal neuralgia. Carbamazapine (tegretol) gives pain relief in 70% of trigeminal and glassophyrangial neuralgia [38].

Neurolytic agents

95% of ethyl alcohol is used to destroy peripheral nerve. Whenever phenol is added to ethyl alcohol, it provides long-term temporary relief. It causes local fibrosis, hence it is not used commonly. Injection of 0.3 ml of glycerol into retrogasserion space for treatment of trigeminal neuralgia resulted 90% patients free of pain. No dysesthesia or anesthesia, dolorosa observed, glycerol acts on demylinated axons assumed to be involved in triggering neuralgia [39].

Uricosuric agents

Probenecid is used in the treatment of gout. It is a uricosuric and renal blocking agent that inhibits reabsorption of urates in the tubules of kidney but increase excretion of uric acid. The urate level in serum is lowered. It induces exacerbations of acute gout. Acute symptoms are controlled by colchicines and therefore the combination is used in chronic gout [40].

Corticosteroids

Systemic corticosteroids are hardly indicated in dentistry but they can be useful for the management of inflammation sometimes. Their use should be restricted for conditions where the correct diagnosis has been made, the dental treatment has been provided sufficiently, no other anti-inflammatory medication has worked and there are no contraindications for their use according to medical history. They should be cautiously used when there are no signs of infection and no possibility of an infection development. Such situations include emergencies like adrenal crisis, anaphylaxis and allergic reactions, severe post-operative swelling, after severe trauma, periapical nerve sprouting and acute apical periodontitis after removal of an acutely inflamed pulp, severe muscle inflammation associated with temporomandibular dysfunction and for some oral ulcerations and mucosal lesions that cannot be managed with topical medications [41].

Some rules of prescribing drugs in pain

1. Prescribe non-opioid analgesics as the FIRST line drugs of pain control for dental procedures.

a. Prescribe combinations of non-steroidal anti-inflammatory drugs (NSAIDs) and acetaminophen following dental procedures where post-operative pain is anticipated unless there are contraindications:

b. Advise patients not to take multiple acetaminophen-containing preparations concomitantly.

2. If use of an opioid is warranted, follow the CDC guidelines: "Clinicians should prescribe the lowest effective dose of immediate-release opioids and should prescribe no greater quantity than needed for the expected duration of pain severe enough to require opioids. Three days or less will often be sufficient; more than seven days will rarely be needed."

a. Prescribe opioids IN COMBINATION with first-line therapy. Avoid multiple acetaminophen containing preparations concomitantly.

b. For adolescents and young adults through 24 years old who are undergoing minor surgical procedures (e.g., third molar extractions), limit opioid prescriptions to 8 to 12 tablets.

c. Codeine and tramadol are contraindicated in children younger than 12 due to variability in metabolism. The use of codeine and tramadol should also be avoided in those aged 12 to 17.

d. Avoid prescribing opioids in combination with benzodiazepines, sedative-hypnotics, or anxiolytics.

3. Dentists should recognize multimodal pain strategies (e.g., ice) for management of acute postoperative pain as a means for sparing the need for opioid analgesics.

4. Educate the patient and family on appropriate use and duration of opioids in a language and at a level (e.g., 8th grade reading level) that they can understand.

a. Review possible adverse effects of opioids, including the sensation of drug craving. Remind them of the dangers of prescription opioid diversion and the importance of secure storage of their medications.

b. Share information on prompt disposal of leftover opioids through community-based drug take back programs, a DEA-approved take back program or FDA guideline for safe disposal of medicine.

c. Advise the patient to avoid combining opioids with benzodiazepines, sedative-hypnotics,

anxiolytics, or other central nervous system depressants, including alcohol. These combinations exponentially (not just additively) increase risk for dangerous respiratory depression.

CONCLUSION

Effective drug regimens can be provided to increase pain control through rational prescription of drug and at the same time education of both the patients and their caregivers which ultimately decrease the unwanted side effects. A comprehensible understanding of pharmacotherapy of drugs is invaluable because thorough knowledge of potential drug interactions can help in designing drug regimes which will be most useful in treating patients with acute or chronic pain. This approach can definitely prevent the stress and anxiety associated with pain. The clinician should develop number of safe and effective analgesic regimens based on assessment of type and intensity of pain.

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Table 1: Acetaminophen and NSAID dosing regimens for dental pain

Drug	Dose (mg)	Frequency	Daily maximum (mg)
Adults			
Acetylsalicylic acid	325–1,000	q4–6h	4,000
Acetaminophen	500–1,000	q4–6h	4,000
Floctafenine	200–400	q6–8h	1,200
Flurbiprofen	50	q4–6h	300

Celecoxib	200	once/day	400
Ibuprofen	400	q4–6h	2,400
Ketoprofen	25–50	q6–8h	300
Ketorolac	10	q4–6h	40 (5 days max.)
Rofecoxib	50	once/day	50 (5 days max.)
Etodolac	200–400	q6–8h	1,200
Diflunisal	500	q12h	1,500
Naproxen	275/250	q6–8h	1,375
Children			
Ibuprofen age 2–12 over age of 12	 10 mg/kg 200–400 mg	 q6–8h q4h	 1,200
Acetaminophe	10–15 mg/kg	q4–6h	65 mg/kg

Table 2: Opioid dosing regimens for dental pain

Drug	Dose (mg)	Frequency	Daily maximum
Adults			
Codeine, with acetaminophen or an NSAID	30–60	q4–6h	240mg
Oxycodone	5–10	q4–6h	
Children			
Codeine, acetaminophen or an NSAID	0.5–1 mg/kg	with q4–6h	3 mg/kg

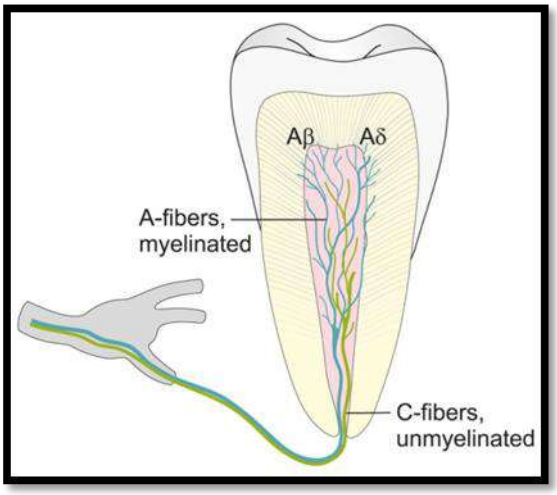

Figure 1. Shows the distribution of intradental A and C fibers.
Unmyelinated C-fibers are in the pulp chamber, whereas myelinated A-fibers are extensively distributed in the pulp dentin border, A-fibres are penetrating the innermost portion of dentin.
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RESEARCH ARTICLE

A Novel Technique for Delivery of Drugs: Microemulsions

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ABSTRACT

Microemulsions are clear, stable and isotropic liquid mixture of oil, water, and surfactant. Mostly they are used in combination with co-surfactants. It basically includes two phases i.e., aqueous phase and oily phase. Aqueous phase contains salts or other ingredients whereas oily phase actually be a complex mixture of olefins and different hydrocarbons. They do not require the high shear conditions because they are formed by the simple mixing of components. As we have observed microemulsions, we observed that they have unique properties like: thermodynamic stability, ultra-low interfacial tension, large interfacial area and the ability to solubilize otherwise immiscible liquids. The major distinction between Microemulsions and emulsions are their transparency, viscosity and more importantly, their thermodynamic stability. Some of the major branches in which microemulsions are widely used are pharmaceuticals, biotechnology, cosmetics, food, environmental detoxification.

Keywords: Microemulsions, environmental, Aqueous, biotechnology, pharmaceuticals

INTRODUCTION

Micro emulsion term also called as transparent emulsion, micellar solution, swollen micelle and solubilized oil, this was first used by Jack H. Shulman in the year 1959 [1]. It may be defined as thermodynamically stable, transparent(translucent) dispersion of water and oil which are stabilized by the interfacial film of the surfactant molecules and the using surfactant is may be pure or the mixture, or combined with a co surfactant like as a medium- chain alcohol (e.g., pentanol, butanol). The normal emulsions are readily different or distinguished from the micro emulsions by its transparency, viscosity, and their thermodynamic stability and ability of form spontaneously [2].

Its system consists of at least 30% of water, 20% of co solvent, at least 30% of oil, 1 to 30% of non- ionic surfactant system having the hydrophilic lipophilic balance, HLB comprised between 9 and 18%, can form spontaneously or thermodynamically stable, that's why the micro emulsion system has theoretically an infinite shelf life under the normal conditions in comparison of the macroemulsions. The size range of the droplets in the microemulsions are remains constant or range between 100-1000 A^0 (10-100nm) [3,4]. These are optically isotropic systems consisting of oil, water and amphiphile. This type of system is beneficial because of their ease of preparation, higher diffusion and absorption rates and for its optical clarity. It shows the excellent solubility properties and its ingredients are effectively overcome the diffusion barrier and penetrate through the layer of skin. Local irritancy may also cause due to vehicles ability of act like penetration enhancers depending on the oil/surfactant constituents.

The attractive formulations for pharmaceuticals are made by the high capacity of microemulsions for drugs. The microemulsion systems are also offer several benefits for oral administrations, increased absorption, improved clinical potency and decreased toxicity [5]. Microemulsions gaining popularity and garnering more attention in recent years because it may enhance the transdermal absorption of drug molecules by modifying their partition coefficients and by increasing its solubilities [6]. Contrasting to the microemulsions, emulsions are unstable systems and without agitation, phase separation will occur in them. There are so many differences in between the emulsion or the microemulsions, one is that the size of the droplets in emulsions are in the range of micrometers, on the other side in microemulsions the size of the micelles in the range of 5-100 nm, they are depending on some parameters like as surfactant type and concentration, the extent of dispersed. Another parameter which is so important because it affects the main characteristics of microemulsion in the presence of electrolytes in the phase i.e., aqueous phase [7].

We are studying about the microemulsions so we also tell the comparison of emulsion and microemulsion if we seen that in contrast to the emulsions, the microemulsion doesn't wantor require the high shear conditions which is generally required in the ordinary emulsions. The microemulsion has contains two basic types and these are Direct (oil dispersed in water, o/w) and reversed (water dispersed in oil, w/o) [8-10]. It improves the transdermal delivery of certain drugs over the different topical preparations such as emulsions [11] and gels [12].

Historical Background

In the mid-1930s, a patent was issued for the combination of water and oil, which was turned into a single-phase system with the help of a third component (surfactant) .The first scholarly study, however, was not conducted until 1943 [13]. Hoar and Schulman demonstrated that spontaneous emulsification can be induced using a powerful surface-active chemical [14]. This is now linked to the creation of microemulsions as a result of the surfactants' promotion of very low interfacial tensions. According to Winsor (1948) there are four types of microemulsions:

(i) Type I- biphasic with an upper excess oil phase and lower O/W emulsion,
(ii) Type II- biphasic with an upper W/O emulsion and lower excess water phase
(iii) Type III- triphasic with upper excess oil phase, middle bi-continuous microemulsion and lower excess water phase
(iv) Type IV- monophasic, single microemulsion phase.

Schulmantitrated a multiphase system (water, oil, and surfactant) with alcohol in 1959 and got a translucent solution they called a "microemulsion. Some researchers used the term 'swollen micelles' [15] to describe these systems at the time, while others used the term 'micellar emulsion' [16]. Microemulsions were formerly confused with Nano emulsions due to similar properties such as a nanometre size range and a similar preparation procedure. A number of research have been published that attempt to distinguish between the two systems [17].

Structure of Microemulsion

Micro emulsions, also known as Micellar emulsions, are dynamic systems in which the interface fluctuates constantly and spontaneously [18]. Oil in water (o/w), water in oil (w/o), and bi-continuous micro emulsions are the structural divisions. Water droplets are dispersed in the continuous oil phase in w/o micro emulsions, whereas oil droplets are

scattered in the continuous aqueous phase in o/w micro emulsions. Bi-continuous micro emulsions can form in systems where the proportions of water and oil are identical [19]. Depending on the quantities of the components, the mixture of oil, water, and surfactants can produce a variety of structures and phases.

Theory

Over the years, various ideas of microemulsion formation, stability, and phase behaviour have been advanced. One reason for their thermodynamic stability is that the surfactant present stabilises the oil/water dispersion, and their creation includes the elastic characteristics of the surfactant film at the oil/water interface, which include the curvature and stiffness of the film as factors. These parameters may have a pressure and/or temperature dependence (and/or the salinity of the aqueous phase) that can be used to infer the microemulsion's stability area, or to demarcate the region where three coexisting phases occur, for example. Calculations of the microemulsion's interfacial tension with a coexisting oil or aqueous phase are also frequently of interest, and they can sometimes be utilised to influence their formation. A simplified thermodynamic rationalisation can be used to explain microemulsion production and stability. The amount of surface tension that a surfactant decreases at the oil–water interface, as well as the change in entropy of the system, may be used to calculate the free energy of microemulsion formation [20].

$DGf = \gamma DA - T\,DS$

Where, DG f =free energy of formation, γ = Surface tension of the oil–water interface DA =Change in interfacial area on micro emulsification, DS = Change in entropy of the system which is effectively the dispersion entropy, and T = Temperature.

It should be emphasised that due to the huge number of nanodroplets generated in a microemulsion, the change in DA is very large. While the value of is always positive, it is extremely small (on the order of fractions of m N/m) and is offset by the entropic component.

Surfactants, co-surfactants and oil used in microemulsion formulation:-

- Surfactants used to stabilize the system; non-ionic, cationic or anionic and zwitter ion.
- The co-surfactant decreases the interfacial tension; -and increase the microemulsion region; like alcohol, amines and cholesterol.
- Oils- hydrocarbon oils such as heptane or -cyclic oils like cyclohexane the droplets i.e., internal phase.

Methods of preparation of Microemulsions

Phase Titration Method

The spontaneous emulsification method (phase titration method) is used to make microemulsions, which can be represented using phase diagrams. The use of a phase diagram to explore the complicated series of interactions that can occur when different components are blended is a good approach. Depending on the chemical composition and concentration of each component, microemulsions and various association structures (such as emulsion, micelles, lamellar, hexagonal, cubic, and various gels and oily dispersion) are generated. The study requires a thorough grasp of their phase equilibrium and the delineation of phase boundaries. As quaternary phase diagram (four component system) is time consuming and difficult to interpret, pseudo ternary phase diagram is often constructed to find the different zones including microemulsion zone, in which each corner of the diagram represents 100% of the particular component. Because constructing a quaternary phase diagram (four component system) takes time and is difficult to comprehend, pseudo ternary phase diagrams are frequently used to locate distinct zones, such as the microemulsion zone, in which each corner of the diagram represents 100% of the component. The region can be separated into w/o or o/w microemulsion by simplyconsidering the composition that is whether it is oil rich or water rich. Observations should be made carefully so that the metastable.

Phase inversion method

Microemulsions undergo phase inversion as a result of the addition of too much dispersed phase or as a result of temperature changes. During phase inversion, significant physical changes occur, including particle size alterations that can affect medication release in vivo and *in vitro*. These approaches work by altering the surfactant's spontaneous curvature. This can be accomplished using non-ionic surfactants by increasing the system's temperature, forcing a transition from an o/w microemulsion at low temperatures to a w/o microemulsion at higher temperatures (transitional phase inversion).The system crosses a point of zero spontaneous curvature and negligible surface tension during cooling, which promotes the generation of finely dispersed oil droplets. The phase inversion temperature (PIT) method is the name given to this procedure. Other characteristics, such as salt content or pH value, may be evaluated instead of the temperature. Changing the water volume fraction can also produce a transition in the spontaneous radius of curvature. Water droplets are created in a continuous oil phase by gradually adding water to the oil. The spontaneous curvature of the surfactant changes from initially stabilising a w/o microemulsion to an o/w microemulsion at the inversion locus when the water volume fraction is increased. At the o/w interface, short-chain surfactants create flexible monolayers, resulting in a bi-continuous microemulsion at the inversion point

Advantages [22]

(i) Microemulsions are thermodynamically stable system and the stability allows self-emulsification of the system.
(ii) A property of microemulsions doesn't depend on the process followed.
(iii) They can solubilize both hydrophilic and lipophilic drugs including drugs, which are relatively insoluble in both aqueous and hydrophobic solvents. Hence, they are called as super solvents. This property of microemulsions is due to the formation of microdomains of different polarity within the same single-phase system.
(iv) The dispersed phase hydrophilic or lipophilic can behave as a reservoir of lipophilic and hydrophilic drugs respectively.
(v) The drug release across a membrane follows pseudo zero order kinetics and it depends on the volume of the dispersed phase, partition of the drug and transport rate of drug across membrane.
(vi) Microemulsions can be sterilized by filtration due to their small size.
(vii) Same microemulsion can carry both lipophilic and hydrophilic drug
(viii) Microemulsions are easy to prepare and require no significant energy contribution during preparation.
(ix) Microemulsions improve the efficacy of a drug, allowing the total dose to be reduced.

Disadvantages [24].

(i) A high surfactant and co-surfactant concentration is required.
(ii) Solubilizing capacity for high-melting compounds is limited.
(iii) To be used in pharmaceutical applications, the surfactant must be nontoxic.
(iv) Environmental factors such as temperature and pH affect the stability of microemulsions. When microemulsions are delivered to patients, these parameters change.

Characterization

It includes physical and chemical tests for oral liquid dosage forms, such as assay, content uniformity, active stability (impurities), appearance, pH, viscosity, density, conductivity, surface tension, size and zeta potential of the dispersed phase, and so on, with regard to the effect of composition on physical parameters.

(i) Differential scanning calorimetry (DSC)
(ii) Polarization microscopy
(iii) Photon correlation spectroscopy (PCS) and total-intensity light scattering (TILS) techniques.
(iv) Static light scattering (SLS), dynamic light scattering (DLS), and small-angle neutron scattering (SANS).
(v) Self-diffusion nuclear magnetic resonance (SD NMR) and small-angle X-ray scattering (SAXS).
(vi) Cross polarizers study.

(vii) Accelerated tests such as centrifugation or freeze thawcycles.

(viii) Furthermore, physical observation upon undisturbed storage at room temperature and refrigerated conditions upon dilution with water to form o/w microemulsions, which can be done by dropwise addition, static serial dilution, or dynamic injection, is used to evaluate the tendency for drug precipitation or crystallisation.

(ix) Centrifugation [25]:Microemulsions systems are subjected to centrifugation at 5000 rpm for 30 minutes and then examined for any phase separation.

(x) Interfacial Tension[26]: The formation and the properties of microemulsion can be studied by measuring the interfacial tension. Ultra-low values of interfacialtension are correlated with phase behaviour, particularly the existence of surfactant phase or middle-phase microemulsions in equilibrium with aqueous and oil phases.

Evaluation

Examination under cross polarising microscope [27,28]: To rule out liquid crystalline systems, the microemulsion systems are examined under a cross polarizing microscope for the absence of birefringence.

Zeta potential measurement [29]: It must be negative or neutral, indicating that the micro emulsion droplets do not have a charge and hence the system is stable. Zeta potential is calculated with Zetasizer. Because electrical charges on particles determine the rate of flocculation, the zeta potential is primarily used for measuring flocculation.

Dilutability Test: To see if the system displays any signs of separation, the Microemulsions are diluted in 1:10 and 1:100 ratios with double distilled water.

Staining Test: Water soluble dye such as methylene blue or amaranth is added in water and microemulsion is prepared with oil and surfactant. A drop of Microemulsions is observed under microscope. Background is found to be blue / red and globule will appear colourless respectively.

Visual Inspection: Visual assessment of microemulsions for homogeneity, optical clarity, and fluidity is possible.

Limpidity Test [30]: The microemulsion's limpidity can be assessed spectrophotometrically with a spectrophotometer.

Assessment of the Rheological Properties [31]: In terms of stability, rheological qualities are crucial. Brookfield digital viscometer can be used to determine it.

Long term stability: The stability of a product can be tested using ICH guidelines. The Microemulsion was held at room temperature for 6 months, and the system was visually inspected and percent transmittance, pH, specific gravity, and rheological evaluation were performed after 1, 3, and 6 months.

Determination of globule size [32]: JDS Quasi Elastic Light Scattering, Uniphase, US Instruments, can be used to determine the globule size of the microemulsion formulation. The size determination is significantly easier with the light scattering approach than with the photomicroscope method.

Determination of thermal stability [33]: Twenty millilitres of drug-loaded microemulsions are stored in a clear borosil volumetric container for one month at three distinct temperatures: 4°, 25°, and 40°C, 1°C in BOD. Periodically, samples are extracted for visual inspection to check for physical changes such as loss of clarity, coalescence, and turbidity, among other things. The samples can also be examined for the loss of aqueous phase, which is a critical component of microemulsion stability.

Study of microstructure of microemulsions [34]: Because it directly provides images at high resolution and can catch any co-existing structure and micro-structural changes, transmission electron microscopy (TEM) is the most essential technology for studying microstructures of microemulsions.

***In-vitro* Skin Permeation Study [33-39]:** Skin permeation study is conducted to find the permeation of drug through skin. The study must be carried out under the guideline compiled by Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA, Ministry of Culture, Government of India). Those microemulsion formulation which is found to be best in evaluation studies is used. The abdominal skins obtained from male Wistar rats weighing 230±20 g (age, 6–8 weeks) is used for in vitro permeation experiments formulations. After hair is shaved carefully with an electric clipper, the skin is excised from the abdominal region of each sacrificed rat and the subcutaneous fat and other extraneous tissues is removed without damaging the epidermal surface. The excised rat skins are washed and examined for integrity, and then stored at 4°C for 24 h in phosphate-buffered saline pH 6.8 (PBS), and then used for the permeation experiments. Franz diffusion cells with excised rat skins with epidermal surface outward are used in the permeation studies. The receptor compartment is filled with 12 ml of PBS and has an effective diffusion area of 3.14 cm2 (20 mm diameter aperture). Throughout the experiment, the diffusion cell was kept at 371°C using a recirculating water bath, and the solution in the receptor chamber was agitated constantly at 600 rpm. A donor chamber is gently filled with the specified amount of formulation. An aliquot of 2 mL sample is removed from the receptor compartment for spectrophotometric analysis at 1, 2, 4, 6, and 8 hours and replaced with an equal volume of fresh PBS. The average cumulative amount of medication penetrated per unit surface area of the skin is displayed vs time using the average values of three readings of in vitro permeation data. The permeation rate of FLZ at steady-state (Jss, micrograms per centimeter per hour) through rat skin is calculated from the slope of linear portion of the cumulative amount permeated through the rat skins per unit area versus time plot. In order to obtain the permeability coefficient Kp (centimeters per hour), the following equation was used **Kp ¼ Jss = C donor** Where, **Kp** is the permeability coefficient**, Jss** is the flux calculated at steady-state, and **C donor** represents the applied drug concentration in the donor compartment.

Nuclear Magnetic Resonance Studies [40-43]: Nuclear magnetic resonance techniques can be used to investigate the structure and dynamics of microemulsions. Self-diffusion measurements utilising several tracer approaches, most often radio tagging, provide information on component mobility. The magnetic gradient on the samples is used in the Fourier transform pulsed-gradient spin-echo (FT-PGSE) technique, which permits simultaneous and quick measurement of the self-diffusion coefficients (in the range of 10^{-9} to 10^{-12} m^2s^{-1}), of numerous components.

Drug release studies: On a modified Franz Diffusion Cell with a volume of 20m L, *In-vivo Drug Release* diffusion research can be performed. Buffer was poured into the receptor chamber. The Microemulsion formulation and the conventional medication solution were kept separate in the donor compartment, which was sealed with cellophane membrane. Samples were taken from the receptor compartment at regular intervals and analysed for drug content using a UV Spectrophotometer set to a certain wavelength. Intestinal membrane within a Franz diffusion cell was used to study *Ex-vivo drug release* into buffer. The donor compartments of two different diffusion cells were filled with microemulsion formulation and plain drug solution, and the temperature of each cell was kept at 37.2°C. By removing samples from the receptorcompartment at predefined time intervals, the amount of medication released from the microemulsion formulation may be measured spectrophotometrically at certain wavelengths.

Applications of Microemulsions

Oral Delivery [44] The development of successful oral delivery systems has always been difficult for researchers because therapeutic efficacy can be limited by gastrointestinal fluid instability or poor solubility. Microemulsions have the potential to improve the solubilization of poorly soluble medicines (especially BCS class II or class IV) and overcome bioavailability issues caused by dissolution. Hydrophilic pharmaceuticals, including macromolecules, can be encapsulated with varied solubility due to the presence of polar, nonpolar, and interfacial domains. These systems protect the inserted medicines from oxidation and enzymatic breakdown while also increasing membrane permeability. Commercially available microemulsion formulations include SandimmuneNeoral(R) (Cyclosporine A), Fortovase(R) (Saquinavir), Norvir(R) (Ritonavir), and others.By increasing the solubility of poorly water-soluble medicines in gastrointestinal fluid, microemulsion formulation has the potential to improve oral bioavailability.

Parenteral Delivery [45]: Lipophilic and hydrophilic medicines have proven difficult to formulate into parenteral dose forms. O/w microemulsions are useful in the parenteral administration of sparingly soluble medicines when suspension administration is not necessary. They offer a way to achieve a relatively high concentration of these medications, which would otherwise necessitate regular administration. Other advantages include a higher physical stability in plasma than liposomes or other carriers, as well as a more resistant internal oil phase to drug leakage. Several medications that are sparingly soluble have been prepared as an o/w microemulsion for parenteral administration. Von Corsewant and Thoren took a different approach, replacing C3-C4 alcohols with parenterally acceptable cosurfactants, polyethylene glycol (400) / polyethylene glycol (660) 12-hydroxystearate / ethanol, while maintaining a flexible surfactant film and spontaneous curvature near zero to achieve a nearly balanced middle phase microemulsion.

Topical Delivery [46]: Topical medication delivery has various advantages over other approaches, one of which is the avoidance of hepatic first-pass metabolism, salivary and stomach drug degradation, and associated adverse consequences. Another is the drug's direct administration and targetability to afflicted skin or eye locations.A number of investigations on drug penetration into the skin have been conducted recently. They can promote the permeability of both hydrophilic (5-flurouracil, apomorphine hydrochloride, diphenhydramine hydrochloride, tetracaine hydrochloride, methotrexate) and lipophilic medicines (estradiol, finasteride, ketoprofen, meloxicam, felodipine, triptolide). Because microemulsion creation necessitates a high surfactant concentration, skin irritation must be considered, particularly when they are meant to be administered for an extended period of time.

Ophthalmic delivery [47-48]: Water soluble medications are administered in aqueous solution in traditional ocular dosage forms, while water insoluble pharmaceuticals are prepared as suspensions or ointments. Low corneal bioavailability and inefficiency in the ocular tissue's posterior portion are two key issues with these systems. The creation of new and more effective distribution systems has been the focus of recent study. Microemulsions have developed as a viable ophthalmic dosage type. Chloramphenicol, an antibiotic used to treat trachoma and keratitis, quickly hydrolyses in ordinary eye drops. Lv et al. looked at a microemulsion made up of Span 20.

Tumour Targeting [49-50] Microemulsions, according to Maranhao, could be used to deliver chemotherapeutic or diagnostic substances to cancer cells while avoiding normal cells.They claimed to have =a higher number of LDL (low density lipoprotein) receptors than normal cells. The microemulsion included a chemotherapeutic medication and had a nucleus of cholesterol esters and not more than 20% triglycerides surrounded by a core of phospholipids and free cholesterol. The lipid portion of low-density lipoprotein (LDL) was comparable in chemical makeup to microemulsions, but the protein portion was missing.When these manufactured microemulsion particles were injected into the bloodstream or treated with plasma, they integrated plasma apolipoprotein E (apo E) onto their surface.Anti-Cancerous drugs' higher concentration could be maintained in the neoplastic cells that have an increased expression of the receptors. Hence, normal tissues and organs could be found safe from the toxic effect of these drugs[50-51].

Nasal Delivery [52]: Microemulsions have recently been investigated as a delivery mechanism for improving drug uptake through the nasal mucosa. In addition, the mucoadhesive polymer helps to extend the time spent on the mucosa. The effect of diazepam on the emergency treatment of status epilepticus was examined by Lianly et al. They discovered that at a dose of 2 mg kg-1, diazepam was rapidly absorbed through the nose, with maximal drug plasma concentration reaching within 2-3 minutes.

Brain Targeting [53-57]: For quick medication delivery to the brain, intranasal administration provides a straightforward, practical, cost-effective, convenient, and non-invasive mode of administration. It permits medications to be delivered directly to the brain, bypassing the brain's barriers. Vyas et al. created a mucoadhesive microemulsion for clonazepam, an antiepileptic medication.The goal was to deliver the drug to the rat's brain quickly. The brain/blood ratio was found to be 2-fold higher at all sampling sites up to 8 hours after intranasal

administration of clonazepam mucoadhesive microemulsion compared to i.v., indicating a broader degree of drug dispersion in the brain.

Cosmetics: Microemulsion formulations lead to faster uptake into the skin. The key factors in the formulation of microemulsions are cost, safety and appropriate selection of ingredients. It contains sodium alkyl sulphate, lecithin, alkyl dimethylamine oxide, propanol, tetra ethylene glycol, mono dodecyl ether, hexadecane, isopropyl myristate[58]. These ingredients are used as surfactants, co-surfactants and oils respectively. The preparation of cosmetic microemulsions is done by emulsion polymerization technique. Ultrafine emulsions can be regarded as thermodynamically unstable because they are o/w emulsion types with the droplet size similar to microemulsion. The ultrafine emulsions are prepared by the method which is known as condensation method that has some advantages in medical products and in the cosmetics. Its droplet size is easily controlled because it has excellent stability and safety properties.

Biotechnology: Microemulsions have a broad scope in various biotechnological processes like enzymatic reactions, immobilization of proteins and bio-separation. Because of the simultaneous solubilization of polar and non-polar reactants in the same solution, the separation of products by physical means and shifting of equilibrium position of the reaction the microemulsions are advantageous over the other multi-phase equilibrium system. The forecast of biotechnological applications has also been reviewed[59,60]. The catalysis reaction has been broadly studied in the microemulsion media. As we know, the use of microemulsions for enzyme catalysis is not arbitrary for enzymes under in-vivo conditions.

Pharmaceuticals: Emulsion forming, liquid crystalline systems are broadly used in the pharmaceutical preparations because of remarkable environment, independent stability, excellent solubilization capacity and easy formation. O/W or W/O types can act as potential reservoirs of Lipophilic or Hydrophilic drugs that can be partitioned between continuous and dispersed phase. Both types of drugs (Lipophilic or Hydrophilic) can be administered together in the same preparation. The use of strongly hydrophobic fluorocarbons (as oils) .

CONCLUSION

Although it took time for researchers to realise the potential of microemulsion as a drug delivery mechanism following its discovery, many studies in the field of pharmaceutics have recently focused on microemulsions. To boost the solubility and bioavailability of medications employed, a variety of hydrophobic, labile pharmaceuticals with poor release characteristics have been synthesised as microemulsions. Before microemulsions to live up to their potential as versatile drug delivery vehicles, however, a significant amount of fundamental work describing their physico-chemical behaviour must be completed. Several research publications on improving medication delivery have recently been published, but more attention on its characterisation, particularly in vitro evaluation, is still needed. Aside from that, research papers show that a higher percentage of surfactant (much higher than CMC level) is used for the formation of microemulsion, regardless of the route of administration, but toxicological evaluation of the prepared microemulsion is lacking, which could be a broad research area in the future.

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Table 1: Common excipients used to formulate microemulsions in recent years.

Oil phase	Surfactant	Co-surfactant
Caprolic acid	Tween 80	Transcutol
Oleic acid	Transcutol	Peg 400
Capmul mcm	Cremophor RH 40	Ethanol
Isopropyl myristate	Cremophor EL	Poloxamer 407
Capryol	Labrasol	Propylene glycol

Table 2: Some commonly used components in Micro-emulsions [23].

Components	Examples	
Oil	MCTs	Gliceryltricaprylate/caprate: Captex 355, Miglyol 810, Neober M-5 etc.
	LCTs	Corn oil, soyabean oil, safflower oil, olive oil etc.
	Mono / Diglycerides	Glicerylcaprylate/caprate (Capmul MCM), Glycerol monooleate (Capmul GMO) etc
	Fatty acids	Oleic acid.
	Propylene glycol ester	Capmul PG-8, Propylene glycol monolaurate (Lauroglycol)
Surfactant	HLB > 10	Tween-20, Tween-80, Polyoxyl 35 castor oil (Cremophore EL), PEG-8 caprylic/ capric glycerides (Labrasol), Polyoxyl 40 hydrogenated caster oil (Cremophore RH 40) etc.
	HLB < 10	Phosphatidylcholine, Unsaturated polyglycolized glycerides (Labrafil M 2125), Span-40, Span-80 etc.
Co-surfactant	Propylene glycol, Polyethylene glycol, Ethanol, Isopropyl alcohol, Isopropyl myristate, ethanol, propanol, isopropanol, etc.	

Table 3. Microemulsion – based products that have completed phase IV clinical trials (60,61)

Year of study	Title of study	Health condition	Drug/Formulation
➢ 002	Cyclosporine A c-2h monitoring versus tacrolimus c-oh monitoring in de novo liver transplant	Liver transplant	Cyclosporine A,tacrolimus , basiliximab, methylprednisolone, prednisone
➢ 003	Evaluation of cyclosporine microemulsion and tacrlimus on the rate new onset diabetes mellitus in kidney	Kidney transplant	Cyclosporine

	transplantation recipients		
➢ 004	Everolimus versus mycophenolate mofetil in combination with reduced dose cyclosporine microemulsion in maintenance heart transplant recipients	Liver transplant	Cyclosporine microemulsion
➢ 004	Efficacy and safety of cyclosporine microemulsion given once a in day	Liver transplant	Cyclosporine microemulsion
➢ 011	Patient with allergic rhinitis due to sensitization pollen	Allergic rhinitis	Lipidic microemulsion, saline
Year of start	**Title of study**	**Health condition**	**Drug/Formulation**
➢ 006	A study to evaluate the efficiency of intravenously administered cyclosporine in de novo liver transplant recipients	Liver transplantation	Cyclosporin

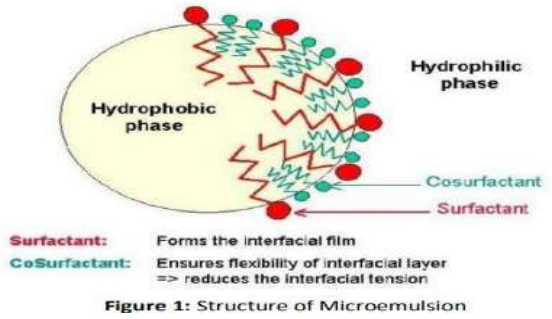

Figure 1 Structure of Microemulsion

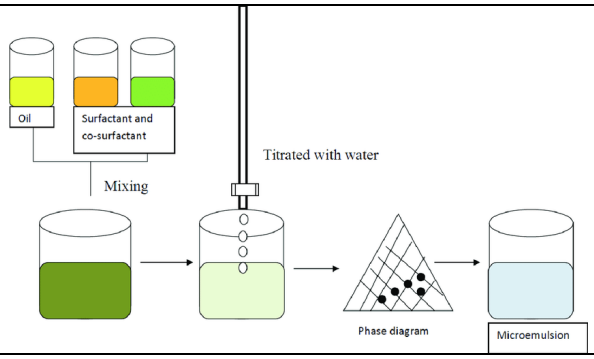

Figure 2. Phase titration method

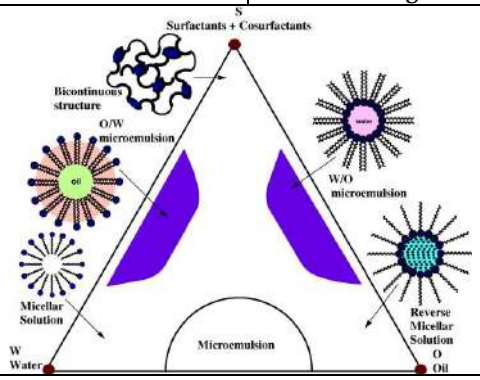

Figure 3. Hypothetical phase region of Microemulsion [21]

REVIEW ARTICLE

Comprehensive Review on Brain Targeting Through Nasal Route

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ABSTRACT

Administration of the drugs from the nose to the brain is considered a viable option to provide a variety of treatments with low bioavailability in the brain to bypass the blood-brain barrier. Absorption of drugs from the sensory area via the vascular route and nerve fibers is the mechanism involved in the delivery of drugs from nose to brain. Nanocarriers containing drugs intended for nasal-to-brain transport have many benefits, including higher patient compliance (Intestinal degradation, BBB, and first-pass metabolism) in addition to overcoming biological barriers (Intestinal degradation, BBB, and first-pass metabolism). Has (compared to injections), increased bioavailability, and rapid absorption of drugs (especially for oil-based drugs) are also provided. This overview covers recent developments in nanocarrier, including drugs such as microemulsions, nanoemulsions, liquid nanoparticles, liposomes, transfer somes, and high molecular weight micelles, and will provide an effective nose to brain drug delivery system. Nasal administration of nanocarriers to the brain has been reported, and various methods are used to overcome mucosal cilia clearance, reduce cerebral blood ratios, and the little number of drugs that can be administered. It also discusses important factors that must be considered in the development of the nose to brain drug delivery system and their toxicity.

Keywords: Brain targeting, Nasal route, Blood-brain barrier(BBB), first-pass metabolism, olfactory pathway.

INTRODUCTION

Oral and parenteral routes are the ultimate preferred route of administration of drugs when systemic consequences are desired [1]. As a result, human oral bioavailability is not surprising. predictions for drugs candidate at the initial phases of discovery and development of drugs targeted [2]. Orally administered drugs enter the bloodstream via the Gastrointestinal system. Although for many drugs, the gastrointestinal system changes depending on pH, enzyme concentration, and other factors[3]. When given this way, medicines that undergo acid hydrolysis or significant liver metabolism may have limited bioavailability. Despite the fact that the primary route of medication delivery provides direct access to the patient, systemic circulation, and maximizes plasma levels, this path can be painful and uncomfortable, and it can only be utilized by trained medical personnel. Upon chronic administration of this route, certain health risks may occur, such as psychological stress, allergies, or shrinkage of the subcutaneous fat at injection location [4]. Alternative drug routes such asbuccal, rectal, transdermal, and nasal are being examined to address issues associated with oral and progenitor routes. All of these routes avoid hepatic first-pass metabolism and offer a different route for systemic drug distribution. This article aims to discuss Intranasal medication delivery techniques that are used, the obstacles to intranasal drug delivery systems, techniques to increase absorption, benefits, and drawbacks of using nasal medication over alternative routes, and the utilization of nasal drug delivery systems. Intranasal medication delivery, which has been in use for millennia, have been given a fresh lease of life [45]. The intranasal drug delivery system has a long history. It was first documented in the Indian healthcare system known as Ayurveda. The "Nasya Karma" is the intranasal drug delivery system that has been used to treat 31 different types of local and systemic diseases [9]. Intranasal medication administration is now most typically used to treat allergic rhinitis and common rhinitis and local inflammation, as well as to relieve nasal congestion [10].

Systemic nasal drug delivery has gained popularity as a possible option in recent years for oral and parenteral drug administration. In Pharmaceuticals & Biopharmaceuticals, the intranasal drug delivery mechanism is examined as a good route for active ingredients. Nasal mucosa is being examined as a possible pathway for achieving quick and a better rate of drug absorptions. The cavity of the nose has an increased area of surface ,, a porous endothelial membrane, high blood flow, and the prevention of hepatic first-pass metabolism are just some of the reasons why scientists are curious about the nasal route promoting due to the great permeability of the nasal mucosa, medicines are delivered systemically. The presence of two physiological obstacles that restrict drug delivery to the blood-cerebrospinal fluid (BCSF) and the Central Nervous System (CNS) and the blood-brain barrier (BBB) makes the a big challenge is getting medications into the brain. (BCSFB). Researchers are interested in the nasal route because it is a practical, predictable, indirect, and safe technique to achieve quick and rapid absorption of drugs. The Nose to Brain technique is well-known because it allows medications to bypass the BBB and flows directly from the nose to the brain and enter the brain via olfactory nerve and trigeminal nerve cell. The olfactory area mucosa of the nose is a direct link between the brain and the nose that is being investigated for CNS-acting medicines. Some drugs, therapeutic proteins, and peptides have enhanced their bioavailability [12]. In recent years, the majority of medications, peptides, and proteins have been delivered efficiently via nose-to-brain delivery. This technique can be used to treat a variety of conditions CNS disorders, such as brain tumors, multiple sclerosis, epilepsy, Alzheimer's disease, psychiatric disorders, and Parkinson's disease. The olfactory and trigeminal regions, as well as systemic circulation, are the most common routes for drugs to reach the brain after intranasal administration. With the help of selected peer-reviewed literature retrieved from several online scientific databases, We looked at recent advances in medication targeting from the nose to the brain, as well as the obstacles that come with it.

Advantages of Nasal drug delivery system [14,15,16,17]

1. Drug degradation doesn't occur in the gastrointestinal tract.
2.The liver does not undergo first-pass metabolism.
3.Drugs can be absorbed rapidly and their effects can be felt quickly.
4. Increasing the larger molecules can be achieved with absorption enhancers and other approaches
5.Drugs with smaller molecular weights have a high bioavailability in the nasal cavity.

6.If a drug cannot be absorbed orally, it can be administered via nasal route for systemic circulation.
7.Research has shown that the nasal route can be as effective as the parenteral route for protein and peptide medications.
8.Comparable to parenteral medication, this form of medication is Patients, especially those on long-term treatment, will find it more convenient.
9. The drug is absorbed through the large nasal mucosa
10. Highly vascularized mucosa facilitates rapid absorption of drugs.
11. The action of onset that occurs quickly.
12. Non-invasive method and ease of administration.
13. The gastrointestinal system and first-pass metabolism are avoided.
14. Bioavailability is improved.
15. Reduction of dosage/reduction of side effects.
16. Self-medication is easier with easy access and needle-free drug application without the requirement for skilled people, which improves patient compliance as compared to parenteral approaches.

Disadvantages of Nasal drug delivery [18]
1.In comparison to the gastrointestinal tract, the nasal cavity has a lesser surface area for absorption.
2.When compared to the oral administration technique, possibilities of irritation.
3.The chemical and components added to the dosage form may produce local side effect and long-term harm to the nasal mucosa cilia.

Limitation [19]
1.Due to mucociliary clearance, Drug can be removed quickly from the nasal cavity.
2.The nasal mucosa is less receptive to drugs with a high molecular weight.
3.Some drugs irritate the nasal mucosa and are destroyed by mucosal enzymes.
4.In comparison to the digestive tract, the area of the surface of the nasal mucosa is quite small. Because of the narrowness of the nasal canal, doses are limited to 25 mg each dose or 20-195 l/per nostril.
5.The most effective nasal delivery of medications to the brain is when the drugs are potent and work at nanomolar or even lower levels in the brain.
6.Pathological disorders such as a cold or an allergy might influence drug absorption.
7.There is no information on the Absorption enhancers used in nasal medicine delivery systems that have been found to be harmful his to pathologically.
8.The cilia on the nasal mucosa can be disrupted by drugs, resulting in local side effects. As well as ingredients incorporated into dosage forms.
9 .Several surfactants used as chemical enhancer are capable of destabilizing and even dissolving membranes when used at high concentrations.
10.A mishandled delivery method could result in mass the dosage form may be mechanically lost in other respiratory areas such as the lungs.

Nasal drug absorption
Mechanism of Nasal Absorption [20]
The medication is absorbed in the nasal cavity and then travels via the mucus membrane. It's the first stage of the absorption procedure. Small, uncharged drugs can readily get over this barrier, whereas larger, charged substances have a hard time doing so. Mucin is the most abundant protein in mucus. It tends to bind to a variety of solutes and inhibits drug molecule diffusion. The mucus layer's structure may change. Due to environment modifications, such as climate changes in pH, and temperature Several absorptions several strategies have been proposed in the past, but only two have been implemented. Mechanisms like these have been utilized a lot.

First mechanism

It's sometimes referred to as para cellular transport. It is a route that transports a lot of water but is slow and passive. In soluble compounds, nasal absorption is inversely proportional to their molecular weights. Bioavailability is reduced when the molecular weight exceeds 1000 Dalton.

Second mechanism [22]

Transport takes place via a lipoidal pathway. It is sometimes referred to as the trans cellular process. Lipophilic drugs are transported by this system, and their lipophilic nature is responsible for their dependence. The medication also travels through a carrier-mediated active transport route to the cell membrane.

The Nasal Cavity: Anatomy and Physiology

Olfaction and breathing are the two fundamental activities of the nasal cavity in humans and animals [23]. However, it also provides important protection as soon as the intake Prior to use, the air is filtered, warmed, and humidified. it reaches the slowest airways. The nasal passages are lined with mucus and hair layers involved in these functions and capture inhaled particles and pathogens. In addition, the resonance of the generated noise, immunological activity, mucous fimbria clearance MMC, and metabolism of endogenous substances are also important functions of the nasal structure [24,25,26]. Two symmetrical halves including four regions (nasal vestibular, atria, respiratory and olfactory regions) are distinguished by anatomical and histological features

Nasal vestibule [27]

Nasal vestibules are located in the most anterior part of the nasal cavity, directly Located between the nostrils, and are around 0.6 cm^2 in size. vibrissae also known as nasal hairs, filters pollutants breathed in this region. On histological examination, This region of the nose is protected by a keratinized epithelium and squamous stratified containing glands of the sebaceous.

Atrium

The atrium is a space that separates the nasal vestibule from the respiratory system. It has stratified epithelium in the front and pseudo stratified columnar cells with microvilli in the rear [28]

Respiratory region [29]

An important part of the nasal cavity is the respiratory system of the nose, also known as the conchae. The nasal cavity is the most important section. And it isseparated into three turbinates that extend from the lateral wall: middle, superior, and inferior. A specialized structure regulates the humidity inbreathed air and the temperature.

Olfactory region [30]

This section is located in the top portion of the nasal cavity and covers the septum and sidewalls. The only part of the central nervous system that functions (CNS)that are accessible to the outside world is the neuroepithelium. Several tiny serous glands create secretions that function as solvents for odorous compounds in this region. The scent allows us to recognize food, partners, and predators provide sensual pleasure, and warn us of danger. This area of approximately 2.5cm^2 contains approximately 50 million primary receptor cells along with cilia.

PATHWAYS FOR DRUG DELIVERY FROM THE NOSE TO BRAIN [12]

The trigeminal nerve pathway and olfactory nerve pathways connect the mucosa of the nose to the brain. A rare kind method for transporting medicines from intranasal delivery.

Olfactory neural pathways

Drugs are released from the nasal cavity, olfactory area to the CSF, or brain parenchyma by way of olfactory neural pathways that run parallel to the nasal olfactory epithelium. Three distinct channels penetrate the olfactory epithelium from the arachnoid membrane covering the subarachnoid space. This pathway is primarily responsible

for transporting lipophilic drug molecules, and the rate of transport is determined by the lipophilic nature of the molecules.

Trigeminal neural pathways

The trigeminal nerves innervate the respiratory epithelium and the olfactory epithelium of the nasal passages before they reach the central nervous system. The olfactory bulbs are where a small fraction of the trigeminal nerve pathways end. The trigeminal nerve's ocular, maxillary, and mandibular divisions send sensory information to the CNS from the mouth cavity, cornea, eyelids, and nasal cavity. The first two are sensory functions, and the third is a combination of sensory and motor functions.

Pathways of the nasal lymphatics and cerebrospinal fluid [31]

Pathways that link the areas of the subarachnoid, CSF, and other parts of the CNS offer a route for medications to be given intranasally. which contains CSF, to the perineurial spaces, which contain olfactory nerves and nasal passages. After being administered intranasally, The CSF, perivascular spaces, and brain intestinal space, transport medicines from the nasal passage, and then are distributed throughout the brain through these pathways. Researchers have discovered that nanotherapeutics are directly transported following intranasal delivery of CSF, without accessing the circulatory system in a major way, which indicates that nasal passages and the CSF have still been established and function.

Factors that influence the absorption of drugs through the nose

1. Drugs related factors
2. Formulation related factors
3. Biological factors

Drugs related factors

The particle size & shape[32]

The shape of particles plays an important impact on drug absorption, with the linear shape of a drug exhibiting lower absorption and the cyclic shape exhibiting higher absorption. the nose to brain targeting should be 5-10 μm because the particle size larger than 10 μm can bedeposited in the nasal cavity.

Molecular weight [33]

The absorptions of medications and their molecular weights up to 300 Da have a linear inverse relationship. When the molecular weight exceeds 1000 Daltons, absorption is greatly reduced. The usage of absorption enhancers can help to improve absorption. Drug absorption is also influenced by the form. Linear molecules absorb less energy than cyclic molecules.

The chemical form of drugs [34]

A drug's chemical form plays a significant role in its absorption. The transformation of medicine into a salt form has the potential to alter its absorptions. The nasal *In situ* absorption of L-Tyrosine carboxylic acid ester has been shown to be much greater than that of Tyrosine.

Polymorphism [35]

It has been discovered that the polymorphic nature of a drug affects the dissolving rate as well as the solubility, which affects drugs absorbed through a biological membrane. It is necessary to select the optimal polymorphism form of the drugs to achieve the desired targeting effects.

Dissolution rate and solubility [36]

Dissolution rate and solubility are the most crucial aspects of powder and solution absorbed by the nose. The type of pharmacological preparations, as well as the solubility of drugs, determine the absorption profile. Because of the nasal cavity's diminutive size, the permissible quantity of medication administration of drugs intranasally should be

minimal. As a result, medications that aren't very water-soluble or need higher dosages may have an impact on the dissolving rate. The particles deposited in the nasal cavity should dissolve before being absorbed. The drug absorption is inhibited if it persists in the form of particles or is washed away.

Factors affecting the formulations

Drug concentration in the formulation

When there is more drug available at the mucosal location, the concentration gradient develops, leading the drug diffusion phenomenon to work and more drugs to be absorbed.

Buffer capacity of the formulation

The nasal formulation is usually given in smaller doses, varying between 25-190 μL. As a result, nasal discharge may change the pH of the dosage. It's possible that the amount of unionized drug accessible for absorption will be impacted. As a result, maintaining the pH in-situ may require a sufficient formulation buffer capacity.

The viscosity of formulation[37]

High-viscosity formulations can interfere with the normal function of the nose, such as ciliary palpation, and mucous pili clearance. Therefore, it is necessary to change the permeability of the drug because the higher the viscosity, the longer the medication is in touch with the nasal mucosa, which in turn increases the time for them to penetrate.

Biological factors

Enzymes

The enzymes cytochrome P450-dependent monooxygenase, carboxylesterase, and aminopeptidase are found in the mucosa of the nose and give pseudo-first-pass effects[38]. The enzymatic barrier protects the lower respiratory airways from toxins, germs, allergens, and other irritants[39].

Mucociliary clearance

The mucociliary transit time in humans is typically measured around 13-15 minutes. Protective barriers[40].Dust, bacteria, and drug particles are trapped by the mucus and they are carried to the nasopharynx and ingested there. As a result, mucous membrane and cilia work together to clear the airways[41].

Nasal blood flow

The mucous membranes of the nose are abundant in vascular and have a significant role in regulating the heat and Inhaled air is humidified. As a result absorption of drugs is based on the constriction and vasodilation of the blood vessels [42].

NOVEL INTRANASAL DRUG DELIVERY SYSTEM TO TARGET CNS

A Nasal Drug Delivery Formulation

Formulations for liquid nasal administration

For intranasal medication delivery to the brain, these are the most often utilized dose formulations. Aqueous formulations make up the majority of them. Because the aqueous dosage form is sensitive to microbial development, preservatives are required in these formulations [43]. Nasal sprays: These are generally used for delivering solutions or suspensions. Because of the accessibility of the dosing actuator and pump, they are capable of delivering precise dosing in the form of fine mist vapors through the nose. [44]Theinjection metering pump's accuracy is determined by the viscosity and surface area of the formula. Special pumps and valves are offered for high viscosity liquids [45].

Nasal formulations for solids

Dry powder for nasal delivery is one of them. Powders for inhalation: For medicines that are unstable in solution or suspension, powdered dosage forms are considered. Preservatives are not required in these dosage forms, and the medicine is relatively stable in these formulations. The effectiveness of the nasal powder formulation is based on the particle's size, the characteristics of aerodynamic flow, and the solubility of the medication that can increase nasal

irritation. Peptide and non-peptide administration can both be accomplished with nasal powders[46]. Nasal powders do not have a regulated dose and might irritate the nose if used incorrectly.

Novel Formulations

There are a variety of novel and nanoformulations which can lead to improved bioavailability issues, such as liposomes, nanoparticles, and microspheres.

Microemulsion

Microemulsion systems are a promising approach for intranasal administration. The microemulsions are stable, clear, and isotropic mixture of water, oil, and surfactants, often combined with co-surfactants [47].Scientists are currently interested in these systems due to their remarkable potential for delivering drugs through a wide range of molecule combinations. As a result, they exhibit the advantages of forming spontaneously, easy production and scalability, increased drug stability and thermodynamic stability, solubility, and bioavailability. Preparation of pharmaceutical dosage forms requires a clear understanding of the structure of the microemulsion, the behavior of phases, the thermodynamic stability is due to several variables., the factor affecting the drug releases from formulations, and the request for the formulation's excipients. Ideal microemulsions, potential uses, and limitations of microemulsions[48].

Nanoemulsion

An oil, surfactant, cosurfactant, and medicine nanoemulsion is an isotropic combination. Miniemulsions, nanoemulsions, ultrafine emulsions, and multiple emulsions are all colloidal sizes between 50 and 100 nm [40]. To the naked eye, these nanoemulsions seem clear and translucent, and they are resistant to sedimentation and creaming. These qualities make nanoemulsions attractive as carriers for basic research and practical applications in a variety of sectors, including chemical, cosmetic, pharmaceutical, and biopharmaceutical fields[49].

Nanoparticles

Nanoparticles are a range of nanoparticles The size range is 11000 nm. Drug molecules' permeability and solubility of poorly soluble drugs have improved[50].This nanoparticle system on the basis of biodegradable polymer is frequently used in the administration of targeted drugs. Because they provide excellentnose enhancement donating to the brain by encasing it. Extracellular transfer of drugs that have been degraded biologically and chemically via Pgp outflow. The system improves the availability of the CNS drug. Polyglycolic acid(PGA), Polylactic acid(PLA), polylactide-coglycolicacids(PLGA), polycaprolactone, and polymethylmethacrylate are known as polymers. Biodegradable, biocompatible, non-toxic[51].

Liposomes

A liposome is a phospholipid vesicle made up of lipid bilayers enclosing aqueous compartments, which can contain drugs and other substances. Liposomal drug delivery methods provide several benefits, including the ability to encapsulate tiny and higher molecules having a wide hydrophilicity range and pH. pKa value some are phospholipid vesicles that include aqueous compartments, one or more that contain medicines and other substance. It is possible to include chemicals. Liposomal medication delivery systems provide several advantages, including a useful both small and huge encapsulation of hydrophilic and Hydrophobic compounds having a wide range of pKa values for hydrophilicity [52]. Researchers found that these compounds enhanced the absorptions of peptides, such as calcitonin and insulin, through their increase in penetration of the membrane[53,54].

PATENTS IN THE FIELD OF DELIVERING DRUGS FROM THE NOSE TO THE BRAIN

Various compounds have been the subject of numerous patent applications, A decades-long investigation has focused on finding ways to deliver drugs to the brain via the nose, and formulas and devices made into delivering the medications directly to the brain. A patent was first filed in 1989 by William Frey H. II, who sought to administer medications through intranasal routes to the brain via the olfactory nerve.

APPLICATIONS

Small molecule delivery to the brain

It's preferable to have a lot of tiny molecules delivered directly as it travels from nose to brain than macromolecules. The delivery of drugs from the nose to the brain is influenced by their size and lipophilic tiny molecules. During 30 minutes the patient was given 7.4nmol of dopamine and nerve growth factor intranasally, an olfactory comparison of concentrations in the rat brains was performed revealed a 5-fold increase in brain dopamine concentration, demonstrating dopamine is more bio available in low molecular weight compounds in the brain than higher molecular weight compounds (NGF)[74,75,76].

Delivery of macromolecules and proteins

Historically, peptides and proteins have had insufficient bioavailability because of their large size and vulnerability to enzyme degradation. Because of their instability and metabolism in the liver, they are normally given intravenously. As a result, intranasal administration looks to have a lot of promise and offers a great platform for brain delivery. Using the extra-nerve pathway, nasal proteins are delivered immediately to the brain. Various types of peptides and proteins are used intranasally to deliver substances to the brain, including insulin, nerve growth factor, and IGF-1[77]. Insulin is best recognized for lowering blood sugar levels, but it also helps with cognition, memory, and learning. Low insulin levels in the CSF are linked to Alzheimer's disease and cause cognitive decline. Insulin increases the synthesis of Insulin Degrading Enzyme (IDE), which then destroys the amyloid-beta beta proteins (A40 and A42) implicated in plaque formation, according to in vitro studies. Insulin also suppresses the activity of a tau kinase involved in neurofibrillary tangle formation [78].

DNA Plasmid Delivery to the Brain

The use of a modified viral vector that can both transduce and over expression of protein with established neuro protective benefits is used in gene therapy for brain disorders. Utilization of viral vectors carries the risk of carcinogenicity and the potential for host immune response and inflammation. Therefore, DNA plasmids were investigated as vectors with the ability to transfect cells after various types of undivided mitosis. After nasal administration, it reaches the brain within 15 minutes, The amount in the brain was found to be 3.9 to 4.8 times greater than in the lungs and spleen[79].

Brain stem cells delivery

Stems cell can be self-renewable and specialize in a variety of different cells type. Stem cells have a strong affinity toward inflammatory and cancerous tissues. Replacement of damaged neurons. When stem cells are administered intranasally, they cross the cribriform plate and travel down the olfactory nerves pathway, the CSF Pathway, and the perivascular pathway to reach various areas of the brain. After intranasal delivery, cribriform plates allow stem cells to pass and go through the pathways of the olfactory nerve, CSF pathways, as well as a perivascular duct to reach various areas of the brain. It was also discovered that spraying hyaluronidase in the nose 30 minutes before injecting stem cells improved stem cell distribution [80].

Aromatherapy as a treatment for psychiatric disorders

Essential oils are used for aromatherapy on the skin or inhaled through the nose. Aromatherapy is a type of therapy that relies on the feeling of scent to help you relax, improve mood, energize the body, and encourage better health. When essential oils are breathed, they release a smell. After passing through the olfactory bulb, the signal is passed to the limbic and hypothalamic systems in the brain. Neurochemical messengers such as serotonin and endorphin are released in these areas of the brain when stimulated. These neurotransmitters connect the nervous system to other body systems, ensuring desirable changes and creating a sense of security. Therefore, Aromatherapy helps treat mental disorder such as stress, insomnia, and depression. For olfactory aromatherapy, candles and diffusers can be used[81,82,83].

The treatment of some major CNS disorders

As shown in Table 4, a few intranasal studies have been conducted to target CNS

CONCLUSIONS AND FUTURE PERSPECTIVES

A significant part of the potential of intranasal delivery is avoiding the BBB and therapeutic delivery of substances in the cells of the brain for treating a wide variety of CNS disorders. Complex nasal anatomy needs to be overcome through the development of even better sensory On the nasal wall, there are devices that prevent medication loss. The olfactory pathways deliver drugs directly into the brain, but a fair amount of them pass through the systemic route, lowering the drug bioavailability in the brain. Hence, a more in-depth analysis is required to build a system for delivering drugs from the nose to the brain that minimizes losses through systemic pathways.

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Table No. 01: The delivery of medicines to the brain through the nose for the treatment of central nervous system illnesses is currently being investigated.

Sl. No.	Disease	Drug	References
01	Sleep disorder	Diazepam, Lorazepam, Alprazolam	55
02	Brain tumor	Telomerase inhibitor GRN163	56
03	Seizures	Oxytocin	57
04	Depression	Venlafaxine	58
05	Alzheimer's disease	Insulin	59
06	Parkinson's disease	Bromocriptine	60
07	Psychosis	Midazolam	61

Table No. 02: Patents Relating to the Brain to Nose drug Delivery Systems.

Sl. No.	Drug	Therapeutic use	The delivery system for drugs	Refs.
01	Modafinil	Narcolepsy and sleep disorders	Microemulsion of lipids	62
02	Deferoxamine	Diseases such as Alzheimer's	In addition to liquid sprays, powder sprays, nasal drops, gel, and ointments	63
03	Retinoic acid	Anosmia		64
04	Diltiazem	Overweight/eating disorders	Liquids, powders	65
05	A variety of growth factors such as fibroblast growth factors and nerve growth factors, as well as GM-1 ganglioside insulin	Depression, Alzheimer's disease, and Parkinson's disease	Lipophilic micelles, liposomes	66
06	polynucleotide	Infection of the CNS, stroke, or cerebrovascular disorder results in nerve damage.		67
07	Recombinant adeno-associated virus (rAAV)	multiple sclerosis, Alzheimer's, Amyotrophic lateral sclerosis,		68
08	A combination of antibodies (IgE ,IgD , IgA , IgM ,IgG)	Huntington's disease, stroke, Alzheimer's disease, Parkinson's	Nasal spray, dry powders, and nasal drops	69
09	The endogenous compounds	Disorders of the nervous system	Sustained-release micro construct made of surface-	70

	(neurotransmitters, neuropeptides, hormones)		Erosion, non-bursting polymers.	
10	Cytokines(Interleukins, Interferons- IFNα, IFNγ, IFNβ,Tumor necrosis factors)	Among auto-immune Illnesses are viral infections such as herpes simplex, viral meningitis, syphilis, as well as gliomas, and multiple sclerosis.		71
11	Epitopes AβPP	Alzheimer's disease	Polymers with dendritic networks can be encapsulated in liposomes or microparticles.	72
12	Antigens and allergens are large molecules.	Immunization	Transferosomes	73

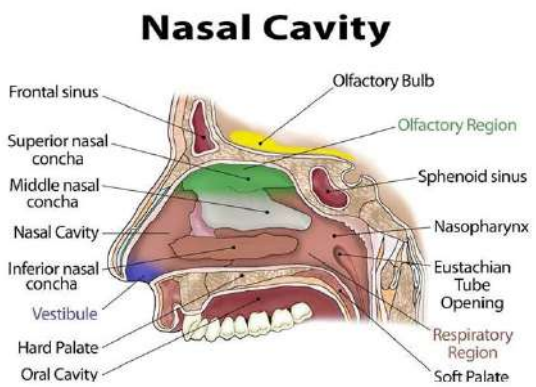

Figure No. 01: Anatomy of Nasal Cavity[6]

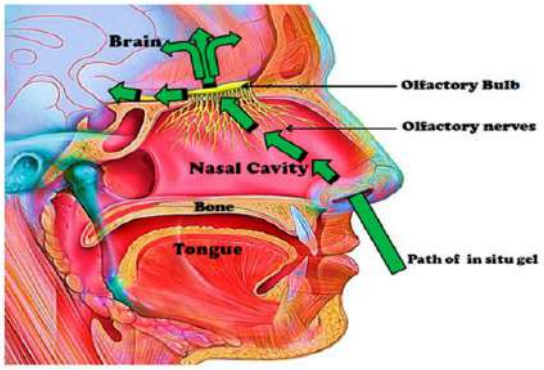

Figure No. 02: Schematic Diagram for Brain Targeting Through Nasal Route[7]

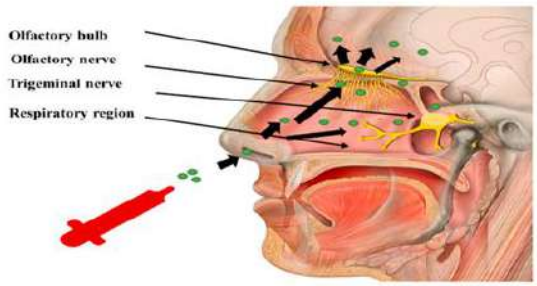

Figure No. 03: Schematic representation of Drug installation to the nasal cavity[8]

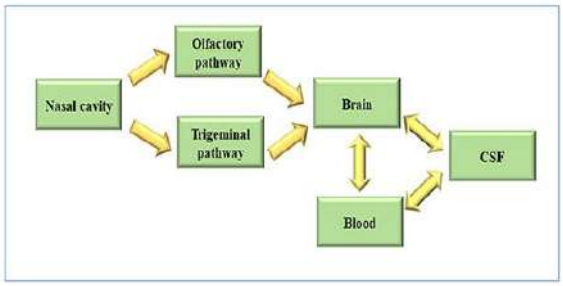

Figure No. 04: Schematic representation for Brain Targeting Through Nasal Route[11]

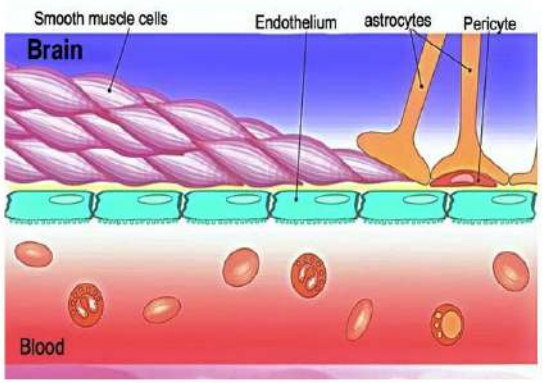

Figure No. 05: Blood-Brain Barrier[13]

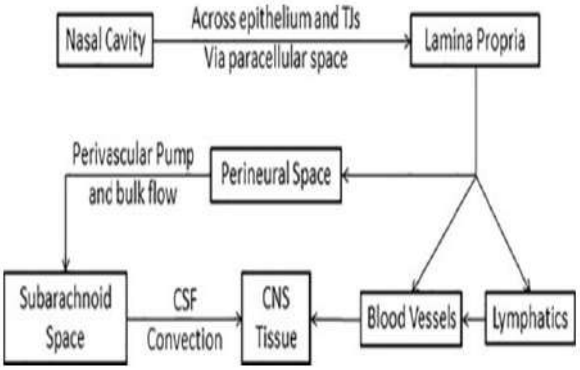

Figure No. 06: Mechanism of Nasal Absorption[21]

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RESEARCH ARTICLE

Phytochemical and Antibacterial Activity of Garlic Extract against Certain Bacterial Pathogens

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ABSTRACT

Phytochemical present in medicinal plant have health benefits and antibacterial activity against on different microbial strains. Ethanolic extract of Garlic (*Allium sativum*) were tested using the agar – well diffusion method for their antibacterial activity against the common bacterial pathogenic *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae*. The susceptibility of the microorganisms to the extract of these plant was compared with each these plant was compared with each other and with selected antibiotics by measuring the diameter of the inhibition zone. The antibacterial activities of these plant were discussed according to the phytochemical components. A qualitative phytochemical analysis was performed for the deletion of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar.

Keywords: phytochemical, Ethanolic extract of Garlic, *Pseudomonas aeruginosa*

INTRODUCTION

Garlic exhibit a broad antibiotic activity against both gram negative and gram positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Rlebsiella*, *Proteus*, *Bacillus*, *Clostridium*, *Helicobacter*, *Pylori*[1] and on diarrheagenic organisms [2]. Cinnamon has been reported to inhibit the growth of several antibiotic resistant strains of bacteria[3] and antibacterial activity of commercial and wild – cinnamon species has been seen[4]. Cinnamon in concentration as low as 0.02% inhibited mold growth and aflatoxin production in yeast extract sucrose broth [5]. The raw juice of garlic was effective against many common pathogenic bacteria, against the strains that have become resistant to antibiotics and even toxin production by some pathogenic strains prevented by garlic [6].

Garlic has an important dietary and medicinal role for centuries. Its therapeutic uses include beneficial effects on the cardiovascular system, antibiotic, anticancer, anti-inflammatory, hypoglycemic, and hormone like effects [7]. Garlic extracts have been used to treat infections for thousands of years [8]. Its typical pungent odor and antibacterial activity depend on allicin, which is produced by enzymatic (alliin lyase) hydrolysis of alliin after cutting and crushing of the cloves [9]. *Allium* vegetables, particularly garlic (*Alluim sativum* Linn.) exhibit a broad antibiotic spectrum against both Gram-positive and Gram-negative bacteria [10]and can be used for formulation of newer spectrum antibacterial substances[11]. Garlic (*Allium sativum*), even from aoristic times, has been used in all parts of the world not only as a spice or a food, but also for treatment of many diseases. Its uses have been inscribed on the walls of ancient Egyptian places of worship and pyramids. Its importance has often been highlighted in the scriptures [12]. The famous herbal doctors. Hippocrates, Paracelsus and Lonicerus recognized garlic as a diuretic, an *emmenagogue*, and used it for the treatment of stomach chills, flatulence, colic etc.[14] observed the inhibitory effect of garlic on pulmonary infection caused by the Mycobacterium avium complex which may be life threatening in non-immunocompromised patients in early stages of immune deficiency diseases such as AIDS.

MATERIALS AND METHODS

Preparation of Extraction

Garlic (*Allium sativum*) was purchase from the super market in Chidambaram. The bulb of garlic was peeled and crushed with pestle and mortar into fine pieces. A bulb of *A. sativum* was used for the preparation of ethanolic extract. Two grams of *A. sativum* cloves were crushed and add 100ml of distilled water and filtered by using whatsmann filter paper No.1. An ethanolicextract of *A. sativum* after filtration was collected and used for futhur investigation.

Phytochemical Study

Garlic (*Allium sativum*) was collected and washed thoroughly in water to remove mud and dust particles. The rhizomes are shade dried and then powdered coarsely in mixer and stored in separate air tight containers at room temperature for further use. The active chemical constituents such as alkaloids, glycosides, terpenoids, flavonoids, reducing sugar, saponins and tannin by the following procedure.

Test for alkaloids

2 ml of extract was taken in a test tube and then 0.2ml dilute HCL was included followed by 1 ml of Meyer's reagent. A yellowish coloration indication alkaloids.

Test for glycosides

Crude extract was mixed with 2ml of chloroform. Then 2 ml of concentration H_2So_4 was added carefully and shaken gently. A reddishbrown color indicated the presence of steroidal ring, i.e., glycose portion of the glycoside.

Test for terpenoids

Cure extract was dissolved in 2 ml of chloroform and evaporated to dryness. To this 2 ml of concentrationH_2So_4 was added and heated for about 2 minutes. A grayish color indicated the presence of terpenoids.

Test for saponins

Cure extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for flavonoids

1 to 5 drops of concentrated hydrochloric acid (HCL) were added to little amount of ethanolic extract of the plant material. Immediate development of a red color indicates the presence of flavonoids.

Test for phenols
Cure extract was mixed with 2 ml of 5% solution of $FeCL_3$. A blue – green or black coloration indication the presence of phenols.

Test for Keller- Kilant test
Cure extract was mixed with 2ml of glacial acetic containing 12 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concertation H_2SO_4. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for Quinones
A small amount of extract was treated with concentrated HCl and observed to the formation of yellow color precipitate.

Test for Tannins
Cure extract was mixed with 2 ml of 5% solution of $FeCL_3$. A blue – green or black coloration indication the presence of tannins.

Antibacterial activity against pathogens
Disc diffusion method was used according to the method previously described by Whatsmann no.1 filter paper disc (6mm) were prepared using a punch machine. Round paper disc were sterilized in autoclave and soaked in 50µl of extract with a concentration of 200mg/ml. Culture of *Escherichia coli*, *Pseudomonas aeruginosa*, ,*Bacillus* sp., *Klebsiella pneumoniae*, and *Staphylococcus aureus* were inoculated into Muller Histon Agar (MHA). Disc were placed on these labelled petri dishes and checked after24 hours incubated at 37◦C for clear zone (mm) around the discs.

RESULTS

Phytochemical analysis of Garlic
The present study reveals that garlic shows the presence of phytochemicals constituents like carbohydrate, sterols, kellerkiliant, Alkaloids and Phenol test. The phytochemical analysis results showed the absent of saponins, Tannins, Flavonoids, Anthocyanin, Reducing sugar, Quinones and Terpenoids. The antibacterial activity of ethanol extract of garlic were evaluated against the bacterial pathogens such as *E. coli*, *P. aeruginosa, Klebsiella* sp., *S. aureus* and *Bacillus* sp. By agar well diffusion method. Table (2) represent the garlic extract against selected bacterial pathogens at different concentration (25µl, 50 µl and 100 µl).

DISCUSSION

According to various garlic and ginger preparations have been shown to exhibit a wide spectrum of antibacterial activity against Gram-negative and Gram-positive bacteria including species of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, *Klebsiella*, *Proteus*, *Bacillus*, and *Clostridium*. Garlic is also rich in anionic components such as nitrates, chlorides and sulfates and other water soluble components found in plants and these components may have antimicrobial properties [15]. Ethanolic extract of sativum was highly effective against all the bacterial species that was taken for the study whereas *Escherichia coli*, *S.typhi* and *S.flexineri* were sensitive to ethanolic extract of ophioscordon. The susceptibility of bacteria to antibiotic chemical is expressed in MIC or high zone of inhibition[17]. The result shows both standard strains of bacteria *S. aureus* and *E. coli* were highly susceptible to different amount of powder garlic extracts. Unlike other solvents chloroform extracts have shown higher inhibition zone against both *S. aureus* and *E. coli*. This was because of their low viscosities which have reciprocal relationship with the rates of diffusion. Thus, the molecule of chloroform extracts of garlic inhibits the bacterial growth. Comparatively the aqueous and ethanol extracts have lower inhibition zone than that of chloroform. The susceptibility of bacterial strains depend on their structural composition, particularly *S. aureus* contain only 2% lipid. So that lipid content of

the membranes will have an effect on the permeability of hydrophobic and volatile bioactive substances in garlic. Hence this phenomenon may favour the destruction of the cell wall and genetic material of *S. aureus* than that of *E. coli*[16]. The extract with higher viscosity inhibits less, because those chemicals have lower diffusion rate to inhibit more bacteria population. That is why an equal amount of 50 mg of chloroform and aqueous show 12 mm and 24 mm zone of inhibition against E. coli, while 100 mg of ethanol and chloroform shows that it inhibits 17 mm and 26 mm of zone of inhibition against *S. aureus* respectively. In the MIC against *S. aureus* is 50 mg of ethanol extract which have an inhibition zone of 12 mm in diameter, this is because the easy penetration of garlic molecules through the lipid membrane present S. aureus. In the case of *E. Coli* the MIC is 50 mg of petroleum ether that have inhibition zone of 10 mm. This is due to *E. coli* is a gram negative bacterium and it have an outer membrane that can make it less susceptible to antimicrobials than gram positive bacteria *S. aureus* [18].

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Table: 1 Phytochemical analysis of ethanol extract of garlic

S.NO	PHYTOCHEMICAL TEST	Ethanol extract
1.	Carbohydrate test	+
2.	Saponins test	-
3.	Tannins test	-
4.	Sterols test	+
5.	Keller Kiliani test	+
6.	Flavonoids test	-
7.	Alkaloids test	+
9.	Anthocyanin test	-
10.	Phenol test	+
11.	Reducing sugar test	-
12.	Quinones test	-
13.	Terpenoids test	-

Present, - Absent

Table: 2 Antibacterial activity of ethanol extract of garlic

Clinical isolation	Zone of inhibition		
	25µl	**50 µl**	**100 µl**
E. coli	10.50±0.21	15.00±0.40	17.00±0.58
P. aeruginosa	13.50±0.70	15.50±0.89	17.10±0.55
Klebsiella sp.	0.00±0.00	7.50±0.50	9.00±0.60
S. aureus	10.30±0.42	12.65±0.45	13.52±0.56
Bacillus sp.	11.00±0.32	13.50±0.55	14.52±0.56

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RESEARCH ARTICLE

Residual Effect on Nutrient Uptake and Yield of Blackgram in Rice - Rice-Blackgram Cropping System Based on STCR-IPNS-Targeted Yield Model

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ABSTRACT

In soil test crop response studies, fertilizer requirement of a crop depend to a larger extend on the native soil fertility. The field experiments were carriedout at the Annamalai University Experimental Farm in Veeranam Ayacut command area. The experimental soil was black, clay loam in texture and belongs to Vertisols and Typic Haplusterts. The technique of inductive methodology was adopted in the present investigation. The main objective of the gradient crop experiment was to create wide variations in soil fertility. After the harvest of gradient crop (rice cv. ADT 43), first test crop rice (cv.ADT 36) was grown during kharif season. Followed by the plots were prepared for transplanting of second test crop of rice (cv.IR 50). Each strip received 24 treatments of different NPK combinations. The blocks received NPK, NPK + FYM, NPK + Azosprillum, NPK + FYM + Azosprillum, respectively. Blackgram (ADT 3) was grown as a residual crop in the same experiment as rice fallow after rice cv.IR50. The results of the experiment revealed that the initial STVs for alkaline KMnO4-N, Olsen-P, neutral normal NH4OAC-K ranged from 235 to 479 kgh-1, 12.95 to 35.40 kg ha-1, and 317 to 490 kg ha-1 from strip I to IV. The mean KMnO4-N values were 289, 351, 388 and 419 kgha-1 in strip I,II,III and IV, respectively. The mean Olsen-P values recorded in strip I, II,III and IV were 17.41,20.76, 22.30 and 27.39 kg ha-1, respectively. The mean values for neutral normal NH4OAC-K were 382,385,409 and 441 kg ha-1 in strip I,II,III and IV, respectively. The pod yield of residual blackgram varied from 419.2 to 635. kgha-1 with mean values of 472.9 (strip I), 508.9 (strip II), 511.7(strip III) and 574.3 kgha-1 in strip IV. The NPK uptake by residual blackgram in treated plot that received fertilizers and organics in previous crop ranged from 21.26 to

46.88, 2.40 to 5.78 and 9.39 to 19.95 kgha-1, respectively. The mean P uptake was found to be 3.24, 3.62, 3.84 and 4.48 kgha-1, respectively. The mean K uptake registered in strip I was 10.78, strip II was 12.35 , strip III was 13.48 and strip IV was 15.15 kgha-1, respectively. This study concludes that application of organic manures with fertilizers plays a vital role in improving soil fertility, productivity and soil health.

Keywords: STCR-IPNS- Residual Blackgram- STVs- NPK uptake, Yield

INTRODUCTION

Black gram is an important pulse crop among the grain legumes grown in India. It contains 24% protein, 60% carbohydrate, 1.3% fat and rich in phosphoric acid. It is commonly known as "urd" or "urd bean". Black gram plays an important role in maintaining and improving soil fertility through its ability to fix atmospheric nitrogen in the soil through root nodules which possesses *Rhizobium* bacteria (Abhitej Singh Shekhawat *et al.,* 2018). About 70% of world's blackgram production comes from India. Blackgram is a warm weather crop and comes up in areas receiving an annual rainfall ranging from 600 to 1000mm. It is mainly cultivated in a cereal-pulse cropping system primarily to conserve soil nutrients and utilize the left over soil moisture particularly, after rice cultivation. Hence, although it can be grown in all seasons, majority of black gram cultivation falls in either rabi or late rabi seasons particularly in peninsular India. Black gram is more tolerant of water logging than moong bean. India is the world's largest producer as well as consumer of blackgram. It produces about 1.5 to 1.9 million tons of urd annually from about 3.5 million hectares of area, with an average productivity of 500 kg per hectare. Targeted yield approach is known as soil test crop response approach. This approach is the basis for optimum resource utilization and balanced crop nutrient management. For a given quantity of yield of any crop, fertilizer requirement can be estimated considering efficiency of soil and fertilizer nutrients. The use efficiencies of nitrogen, phosphorus and potassium are 30–50%, 15–20%, and 60–70%, respectively . The use of an appropriate combination of organic, inorganic and bio- fertilizers, depending on soil fertility status is a step forward for providing balanced fertilization to crops, such integrated nutrient management (INM) can increase the income of farmers.

MATERIALS AND METHODS

The field experiments were carriedout at the Annamalai University Experimental Farm in Veeranam Ayacut command area. The experimental soil was black, clay loam in texture and belongs to Vertisols and *Typic Haplusterts.* The technique of inductive methodology was adopted in the present investigation, the main objective of gradient crop experiment was to create wide variations in soil fertility of the experiment, after the harvest of gradient crop (rice cv. ADT 43), first test crop rice (cv.ADT 36) was grown during *kharif* season. Followed by the plots were prepared for transplanting of second test crop of rice (cv.IR 50). Each strip received 24 treatments of different NPK combinations. The blocks received NPK, NPK + FYM, NPK + *Azosprillum*, NPK + FYM + *Azosprillum*, respectively. Blackgram (ADT 3) was grown as residual crop in the same experiment as rice fallow after rice cv.IR50. Treatment consisted of 5 levels of N(0,50,100,150&200 $kgha^{-1}$), 4 levels of P_2O_5(0,30,60 &90 $kgha^{-1}$),3 levels of K_2O $kgha^{-1}$ (0,40 & 80 kg ha^{-1}), 2 levels of FYM (0, 12.5 tha^{-1}) , 2 levels of *Azospirillum* (0,2 $kgha^{-1}$) . Blackgram (ADT 3) was grown as residual crop in rice-rice- blackgram cropping sequence in Vertisols soil. The treatment structure is given in figure 1. After the harvest of the second test crop of rice cv. IR20. the initial sol samples were collected and analyzed for their available NPK. The results served as the initial soil test values for the residual blackgram .The residual effect on yield and NPK uptake by blackgram also estimated and registered.

RESULT AND DISCUSSION

The residual effect of the above said treatments on initial soil NPK staus, yield and NPK nutrients uptake by blackgram were registered in table 2.

Initial soil available nutrients

The available nitrogen, phosphorus and potassium values ranged from 235 to 479 kg ha^{-1}, 12.95 to 35.40 kg ha^{-1} and 317 to 490 kg ha^{-1}, respectively from strip I to IV. The mean soil available nitrogen values were 289, 351, 388 and 419 kg ha^{-1}. Regarding the soil available phosphorus, the mean values recorded in strip I,II,III and IV were 17.41, 20.76, 22.30 and 27.39 kg ha^{-1}. The mean values for neutral normal ammonium acetate potassium were 382, 385, 409 and 441 kg ha^{-1}in strip I,II, III and IV, respectively. In the control plots, the available nitrogen, phosphorus and potassium values ranged from 172 to 289 kg ha^{-1}, 8.05 to 19.81 kg ha^{-1} and 287 to 393 kg ha^{-1}, respectively. The mean values of soil available nitrogen, phosphorus and potassium status were 224 kg ha^{-1}, 14.50 kg $ha^{-1}$and 338 kg ha^{-1}, respectively. The results are conformity with the findings of santhi (1995) in rice-rice-pulse cropping sequence in *Typic Ustropept*. An improvement in available nutrient status of the soil with the incorporation of chemical fertilizer, biofertilizer and organic manure could be attributed to conserved soil nitrogen and increased availability of other nutrients as being its constituent as well as mineralize from the native source in soil. The results of present investigation are in line with the finding of Parthasarathi Jangir et al., 2017.

Grain yield

The grain yield or residual blackgram varied from 419.2 to 635.6 kg ha^{-1} with mean values of 472.9 kg ha^{-1} in strip I, 508.9 kg ha^{-1} in strip II, 511.7 kg ha^{-1} in strip III and 574.3 kg ha^{-1} in strip IV. The highest grain yield of 636.5 kg ha^{-1} was registered in the treatment plot wherein 200- 90-40 kg ha^{-1} (N-P2O5-K2O) was applied in the previous season with rice as test crop along with FYM @ 12.5 tonnes ha^{-1} and *Azospirillum* @ 2 kg ha^{-1}. The corresponding initial soil available nitrogen, phosphorus and potassium status were 479-35.16-480 kg ha^{-1}, respectively in strip IV. The lowest grain yield of 419.2 kg ha^{-1} was recorded in strip I, which was received 50 kg N and FYM @ 12.5 tonnes ha^{-1} in the previous season. The associated initial soil available nitrogen, phosphorus and potassium status were 235- 12.95 – 326 kg ha^{-1}, respectively. Among the control plots, highest grain yield (468.1 kg ha^{-1}) was recorded in strip IV and the lowest grain yield (395.6 kg ha^{-1}) was recorded in absolute control plot of strip I. The yield increase could be attributed to the synergistic effects due to application of fertilizers + FYM and resultant increase in fertilizer use efficiency (Roy, 1994). IPNS had a profound influence on the grain yield of blackgram. Similar results have been reported by Santhi and Selvakumari (1999). As both inorganic fertilizers, organic manures and bio-fertilizers provides readily availability of essential nutrients to crop plant thereby helps in enhancing yield attributes because optimum utilization of solar light and its conversion to starches through photosynthesis resulted higher grain number and weight that resulted in increased seed yield. Our results are in conformity with Patil *et al.,* (2014).

Nutrients uptake

The nitrogen, phosphorus and potassium uptake by residual blackgram in treated plots that received fertilizers and organics in previous crop (rice cv. IR 50), ranged from 21.26 to 46.88 kg ha^{-1}, 2.40 to 5.78 kg ha^{-1} and 9.39 to 19.95 kg ha^{-1}, respectively. The mean nitrogen uptake by residual blackgram registered in strip I,II,III and IV were 30.44, 31.49, 32.23 and 33.87 kg ha^{-1}, respectively. The mean phosphorus uptake was found to be 3.24 kg ha^{-1} (strip I), 3.62 kg ha^{-1} (strip II) , 3.84 kg ha^{-1} (strip III) and 4.48 kg ha^{-1}(strip IV), respectively. The mean potassium uptake recorded in strip I was 10.78 kg ha^{-1}, strip II was 12.35 kg ha^{-1} , strip III was 13.48 kg ha^{-1} and 15.15 kg ha^{-1} in strip IV. The highest nutrient uptakes of residual blackgram were obtained due to higher dry matter production and increased availability of nutrients from the INM might have enhanced NPK uptake. These results were in conformity with the findings of Geetha and Velayutham (2016).

CONCLUSION

From the result narrated above it is concluded that application of 200- 90-40 kg of N-P_2O_5-K_2O ha^{-1} + FYM @ 12.5 t ha^{-1} + *Azospirillum* @ 2 kg ha^{-1} to proceeding rice crop has established a significant residual effect on NPK uptake and yield (636.5 kg ha^{-1}) of blackgram besides maintaining soil available nutrients and observed to be an effective nutrient management package for rice- rice- blackgram sequence. It is therefore suggested and recommended that combination of inorganic,organic and bio - fertilizers should be adopted by farmers to sustain yield of blackgram and soil health in Vertisols.

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Table 1: Residual effect of NPK fertilizers, organic manure and bio-fertilizer on initial soil available nutrients, grain yield and nutrient uptake by blackgram cv. ADT 3 in STCR – IPNS experiment.

S. No.	Soil and Plant Parameters	Strip I		Strip II		Strip III		Strip IV	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
1.	$KMnO_4$-N (kg ha^{-1})	235-358	289	273-418	351	321-463	388	350-479	419
2.	Organic –C (g kg^{-1})	5.90-7.60	6.46	5.90-7.20	6.57	6.65-7.95	7.38	6.25-7.95	7.00
3.	Olsen-P (kg ha^{-1})	12.95-24.26	17.41	14.70-26.03	20.76	16.42-30.14	22.30	19.93-35.40	27.39
4.	NH_4OAc-K (kg ha^{-1})	317-449	382	324-461	385	372-470	409	409-490	441
5.	Grain Yield (kg ha^{-1})	419.2-525.0	472.9	446.6-559.9	508.9	452.3-568.7	511.7	479.4-635.6	574.3
6.	N Uptake (kg ha^{-1})	21.26-41.58	30.44	22.97-42.71	31.49	23.37-44.68	32.23	24.35-46.88	33.87
7.	P Uptake (kg ha^{-1})	2.40-4.22	3.24	2.87-4.26	3.62	3.14-4.73	3.84	3.17-5.78	4.48
8.	K Uptake (kg ha^{-1})	9.39-13.92	10.78	9.75-15.10	12.35	9.78-17.46	13.48	11.02-19.95	15.15

Table 2: Range and mean values of initial soil available nutrients, grain yield and nutrient uptake in the residual crop experiment with blackgram cv. ADT 3 under STCR – IPNS.

S. No.	Soil and plant parameters	Strip I to IV		Strip I to IV	
		Treated Plots		Control Plots	
		Range	Mean	Range	Mean
1.	$KMnO_4$-N (kg ha^{-1})	235 – 479	362	172- 289	224
2.	Organic carbon (g kg^{-1})	5.90 – 7.95	6.85	5.60 – 6.95	6.20
3.	Olsen-P (kg ha^{-1})	12.95 – 35.40	21.97	8.05 – 19.81	14.50
4.	NH_4OAc-K (kg ha^{-1})	317 – 490	404	287 – 393	338
5.	Grain yield (kg ha^{-1})	419.2 – 635.6	517.0	395.6 – 468.1	429.7
6.	N uptake (kg ha^{-1})	21.26 – 46.88	32.01	15.85 – 20.41	17.96
7.	P uptake (kg ha^{-1})	2.40 – 5.78	3.80	2.10 – 3.13	2.58
8.	K uptake (kg ha^{-1})	9.39 – 19.95	12.94	8.95 – 10.75	9.58

Strip I		Strip II		Strip III		Strip IV		
$N_2P_2K_0$	$N_3P_2K_2$	$N_3P_3K_1$	$N_0P_0K_0$	$N_4P_3K_2$	$N_4P_3K_1$	$N_3P_3K_2$	$N_3P_3K_0$	NPK
$N_2P_2K_2$	$N_2P_2K_1$	$N_2P_0K_0$	$N_1P_1K_0$	$N_0P_0K_0$	$N_3P_0K_0$	$N_0P_0K_0$	$N_1P_0K_0$	
$N_2P_1K_1$	$N_0P_0K_0$	$N_4P_2K_2$	$N_3P_1K_1$	$N_2P_0K_1$	$N_2P_1K_0$	$N_1P_1K_1$	$N_4P_4K_1$	
$N_3P_1K_1$	$N_1P_0K_0$	$N_0P_0K_0$	$N_4P_3K_1$	$N_2P_2K_2$	$N_1P_1K_1$	$N_4P_2K_2$	$N_2P_2K_1$	NPK + FYM
$N_0P_0K_0$	$N_3P_3K_1$	$N_3P_3K_0$	$N_4P_2K_1$	$N_3P_2K_2$	$N_2P_0K_0$	$N_4P_3K_2$	$N_3P_0K_0$	
$N_1P_1K_0$	$N_2P_0K_1$	$N_2P_1K_0$	$N_2P_2K_0$	$N_2P_3K_2$	$N_0P_0K_0$	$N_2P_1K_1$	$N_0P_0K_0$	
$N_3P_0K_0$	$N_0P_0K_0$	$N_3P_3K_2$	$N_2P_0K_1$	$N_3P_3K_1$	$N_4P_2K_2$	$N_3P_2K_2$	$N_0P_0K_0$	NPK + *Azospirillum*
$N_4P_3K_1$	$N_2P_1K_0$	$N_0P_0K_0$	$N_2P_1K_1$	$N_2P_2K_1$	$N_0P_0K_0$	$N_2P_2K_2$	$N_1P_1K_0$	
$N_2P_0K_0$	$N_1P_1K_1$	$N_4P_3K_2$	$N_1P_0K_0$	$N_3P_3K_0$	$N_4P_2K_1$	$N_3P_1K_1$	$N_2P_2K_0$	
$N_4P_2K_1$	$N_3P_3K_2$	$N_2P_2K_1$	$N_2P_2K_2$	$N_0P_0K_0$	$N_2P_1K_1$	$N_0P_0K_0$	$N_2P_0K_1$	NPK + FYM + *Azospirillum*
$N_3P_3K_0$	$N_4P_2K_2$	$N_3P_2K_2$	$N_0P_0K_0$	$N_2P_2K_0$	$N_1P_1K_0$	$N_4P_3K_1$	$N_2P_1K_0$	
$N_0P_0K_0$	$N_4P_3K_2$	$N_1P_1K_1$	$N_3P_0K_0$	$N_1P_0K_0$	$N_3P_1K_1$	$N_2P_0K_0$	$N_3P_3K_1$	

Figure 1. Field layout: STCR – IPNS on rice- rice- blackgram cropping system (Treatment Structure)

RESEARCH ARTICLE

Gene action and Combining Ability Studies for Grain Yield and its Related Traits in Black gram (*Vigna mungo* L.)

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ABSTRACT

Identification of Superior genotypes from variability generated through hybridization. L x T mating Design was conducted with seven lines and three tester's helps to understanding the nature of gene action and combining ability controlling grain yield and related characters are resolvable for black gram varietal improvement. The Observation were taken for ten quantitative characters viz., days to 50 per cent flowering, plant height at maturity, number of branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, 100 seed weight and seed yield per plant were collected on 21 hybrids and their 10 parents. Based on *per se* performance and *gca* effect, the lines L_3 and T_2 were adjudged as the best for most of the traits studied. Among the hybrids, L_4 X T_1 exhibited high *sca* effect for most of the economic traits. The hybrid L_2 X T_2 and L_6 X T_1 showed desirable performance based on *per se* and *sca* for seed yield and its attributing characters and so this hybrid could be exploited for further crop improvement. The result indicated that the general combining and specific combining ability variance for all the characters measured signifying the prominence of both additive and non additive genetic components in the present study.

Keywords: Line X Tester, Combining ability, GCA, SCA, non-additive gene action and Vigna mungo

INTRODUCTION

Black gram (*Vigna mungo* (L.) Hepper) is third most important pulse crop in Indian agriculture after chickpea and red gram. It is grown in various agro-ecological conditions and season under diverse cropping system. In India, it is covered an area of about 3.75 million hectares with the production of 3.23 million tonnes. The major producing states are Madhya Pradesh, Uttar Pradesh, Rajasthan, Maharashtra and Andhra Pradesh In Tamil Nadu, black gram covers an area of about 2.12 lakhs acre with production of 8.08 lakhs quintal and productivity 700 kg per ha[1]. Andhra

Pradesh is one of the leading blackgram growing states of India with an area of 3.81 kg lakh hectars, production of 3.13 lakhs tonnes and productivity of 821 kg/ ha. It dried and edible grains contains rich in protein (25-26 %) which is almost three time higher than cereals and also rich in carbohydrates (60%), fat (1.5%), minerals (2.3%), vitamins and amino acids. It also contributes a major source of lysine content in the vegetarian diet and good source of vitamins like thiamine, niacin, riboflavin and also rich in essential iron and phosphorous content in the grain. In a self pollinated crop like black gram where pure line breeding is a thumb rule, crosses with high sca effects can be utilized to isolate desirable transgressive segregants which mat result in an outstanding variety. Hence development of short duration and high yielding varieties that suited well into different cropping is highly essential for breaking the yield barrier in black gram. The low productivity of black gram can be further attributed to unavailability of high yielding varieties with good plant type and disease resistance [2]. Nature of gene action controlling economic traits plays important role in crop improvement programme and the exploring the gene action is essential [3]. Gene action is helpful for selection of breeding methods for improving the specific character only additive type of gene action is fixable and essential to develop stable genotypes[4]. Genetic variability study includes the parameter such as phenotypic, genotypic and environmental coefficient of variation, heritability and genetic advance are undeniable needed to start an efficient breeding method [5]. Combining ability study utilizing L x T analysis provides information on genetic component of variation. The study GCA helps for selection of best genotypes with additive gene action and the SCA are helpful for selection of superior hybrid combination. Considering all these criteria the present investigation was aimed to identified best parents and crosses for high yield and short duration traits in black gram.

MATERIALS AND METHODS

The parents used for the study were collected from NPRC-Vamban, Tamilnadu Agricultural University, Coimbatore and NRI-Agritech, Guntur. Among these ten parents seven were used as lines namely CB-P.133/3 (L_1), CB-P.133/14 (L_2), CB-P.28 (L_3), CB-P.11 (L_4), CB-P.133/52 (L_5), CB-P.47 (L_6) and CB-P.132 (L_7) and three testers VBN-8 (T_1), Nandhi (T_2), MDU-1 (T_3).The seven lines and three testers were crossed in a line x tester mating design resulting in twenty one hybrids obtained. Unpaired sowing of seven lines and three testers were taken up during June 2020. The seeds were sown in a spacing of 30 x 10 cm by forming a ridges and furrows at five days interval for synchronizing the flowering. Thinning was done on the tenth day after planting to maintain effective plant population. During October 2020, the seeds of twenty one F_1 hybrids obtained through Line X Tester mating design were sown in the field along with parents. The experimental data were collected and analyzed for combining ability and gene action. The genotype VBN-8 was used as standard check variety. The ten parents and 21 hybrids were sown in a Randomized Block Design (RBD) with three replications. Recommended agronomical practices were followed and need based plant protection measures were also adopted to raise the crop. The biometrical observation were taken on ten randomly selected plants for in each replication for ten quantitative traits *viz.,* days to 50 per cent flowering, plant height at maturity, number of branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, 100 seed weight and seed yield per plant.

RESULT AND DISCUSSION

The combining ability variance was studied for all the ten characters and furnished in Table 1. The variance due to hybrids and line × tester were significant for all the ten characters studied. The variances due to lines and testers were also significant for all the ten traits. Therefore further analyses were appropriate.. The estimates of GCA and SCA variances revealed that the SCA variance was higher than GCA variance for all the ten characters analyzed. It's indicating the importance of non additive gene action. The ratio of GCA to SCA variance was less than unity for all the traits studied, indicating the preponderance of non-additive gene action governing the traits. The similar results were reported by [6], [7],[8], [9] and [10]. The GCA effect is due to additive gene action and is fixable [11].The specific combining ability is the deviation from the performance predicted on the basis of general combining ability [12]. The

specific combining ability is controlled by non- additive gene action. The *sca* effects are important criteria for the evaluation of hybrids[11]. Based on *gca* effect, the line L_3 ranked first since they had significant *gca* for plant height, number of branches per plant, number of clusters per plant, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length and seed yield per plant. The line L_1 was considered as the next best for plant height, number of clusters per plant, number of pods per plant, number of seeds per pod, pod length and 100 seed weight. (Table 2).The *sca* effects are important criteria for the evaluation of hybrids. High *sca* effects alone may not be appropriate choice of the hybrid for heterosis exploitation because the hybrids with low mean value may also possess high *sca* effects, if *gca* effects of the parents were very low or even negative (Grakh and Chaudhary, 1985)[13]. It observed from the present study the hybrid $L_2 \times T_2$ and $L_6 \times T_1$ were adjusted as the best for seven out of ten characters studied. The hybrid $L_2 \times T_2$ was best for days to 50 per cent flowering, plant height, number of pods per cluster, number of pods per plant, number of seed per pod, 100 seed weight and seed yield per plant. The hybrid $L_6 \times T_1$ was best for plant height, number of pods per cluster, number of pods per plant, number of seeds per pod, pod length, 100 seed weight and seed yield per plant. The hybrid $L_3 \times T_3$ was adjusted as the best for five out of ten characters. The hybrid $L_3 \times T_3$ was best for number of branches per plant, number of pods per cluster, number of pods per plant, pod length and seed yield per plant (Table 3). The employing hybridization techniques in pulses are very difficult because the flowers are very small and delicate with cleistogamous in nature. It practically observed that through hand emasculation and pollination techniques is less than 5% seed set is possible. Hence, heterosis could be favorably exploited only if proper male sterility system is available.

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Table 1. Analysis of variance for combining ability in black gram for yield and its component traits

Source	D.f.	Mean square									
		Days to 50 per cent flowering	Plant height at maturity	Number of branches per plant	Number of clusters per plant	number of pods per cluster	Number of pods per plant	Number of seeds per pod	Pod length	100 seed weight	Seed yield per plant
Replication	2	0.004	0.86	0.03	0.05	0.001	0.02	0.006	0.01	0.001	0.01
Hybrids	20	20.00**	33.75**	1.77**	79.55**	0.85**	302.51**	1.43**	0.45**	0.46**	9.46**
Line	6	48.07**	27.88**	1.84**	104.08**	1.25**	484.32**	2.39**	0.73**	0.70**	9.06**
Tester	2	31.95**	204.71**	3.77**	422.16**	2.05**	664.45**	4.76**	1.39**	0.67**	6.56**
Line x Tester	12	3.97**	8.19**	1.39**	10.19**	0.45**	151.28**	0.39**	0.15**	0.30**	10.14**
GCA		0.42	0.67	0.01	1.81	0.01	3.94	0.03	0.01	0.004	0.02
SCA		1.32	2.19	0.46	3.39	1.15	50.42	0.13	0.04	0.10	3.37
GCA/SCA		0.32	0.31	0.02	0.53	0.01	0.08	0.23	0.25	0.04	0.01

** Significant at 1 per cent level

Table 2. General combining ability effects of parents for different traits in black gram

Traits / Genotypes	Days to 50 per cent flowering	Plant height at maturity	Number of branches per plant	Number of clusters per plant	number of pods per cluster	Number of pods per plant	Number of seeds per pod	Pod length	100 seed weight	Seed yield per plant
L_1	4.43**	-3.18**	-0.40**	0.35 **	-0.26 **	4.41 **	0.50 **	0.13 **	0.16 **	-0.04
L_2	-0.14*	1.46**	-0.23**	0.28 **	-0.22 **	-3.37 **	0.55 **	0.29 **	0.22 **	-0.52 **
L_3	0.76**	-0.90*	0.38**	0.68 **	0.63 **	7.58 **	0.37 **	0.39 **	-0.38 **	2.12 **
L_4	-0.75**	1.26**	-0.36**	0.66 **	0.33 **	10.29 **	-0.74 **	-0.32 **	-0.03 **	0.22 **
L_5	0.36**	1.06*	0.84**	-3.09 **	0.12 **	-8.81 **	0.12 **	-0.10 *	0.40 **	-0.29 **
L_6	-2.01**	-1.03*	-0.20**	-3.60 **	-0.25 **	-4.91 **	-0.28 **	-0.35 **	-0.09 **	-0.87 **
L_7	-2.67**	1.32**	-0.03*	-1.29 **	-0.36 **	-5.19 **	-0.51 **	-0.05	-0.28 **	-0.61 **
T_1	1.37**	-3.34**	-0.42**	0.41**	0.31**	0.16**	-0.46**	-0.28**	0.20**	-0.45**
T_2	-1.03**	2.85**	0.43**	0.42**	-0.32**	5.54**	-0.03*	0.22**	-0.14**	0.63**
T_3	-0.34**	0.48	-0.01	-4.68**	0.01	-5.70	0.49**	0.06*	-0.07**	-0.17**
SE (lines)	0.05	0.42	0.013	0.05	0.012	0.05	0.02	0.04	0.004	0.04
SE (Testers)	0.03	0.28	0.009	0.03	0.01	0.03	0.02	0.03	0.002	0.02

* Significant at 5 per cent level ** Significant at 1 per cent level

Table 3. Specific combining ability effects of hybrids for yield and yield attributing traits in black gram

Traits / Genotypes	Days to 50 per cent flowering	Plant height at maturity	Number of branches per plant	Number of clusters per plant	number of pods per cluster	Number of pods per plant	Number of seeds per pod	Pod length	100 seed weight	Seed yield per plant
L_1 X T_1	-0.37**	0.53	0.66**	-0.12	-0.01	0.61 **	0.01	0.01	-0.13 **	2.24 **
L_1 X T_2	1.54**	-1.66**	0.56**	1.07 **	-0.32 **	-2.94 **	0.06	0.11	0.01	-0.45 **
L_1 X T_3	-1.17**	1.12	-1.22**	-0.95 **	0.32 **	2.33 **	-0.07	-0.11	0.12 **	-1.79 **
L_2 X T_1	1.47**	-0.27	-0.19**	2.43 **	0.14 **	3.48 **	-0.46 **	0.08	-0.20 **	-1.05 **
L_2 X T_2	-0.91**	-1.82*	0.04	-0.88 **	0.37 **	2.08 **	0.29 **	-0.24 **	0.21 **	1.55 **

L_2 X T_3	-0.56**	2.09**	0.16**	-1.54 **	-0.51 **	-5.56 **	0.17 **	0.16 *	-0.01	-0.51 **
L_3 X T_1	-0.09	-0.45	-0.20**	-0.21 *	-0.56 **	-11.71 **	0.06	-0.29 **	0.47 **	-3.04 **
L_3 X T_2	-0.67**	0.01	0..02	1.21 **	0.01	-1.78 **	-0.11*	-0.03	-0.19 **	1.15 **
L_3 X T_3	0.76**	0.44	0.18**	-0.99 **	0.54 **	13.48 **	0.05	0.33**	-0.28 **	1.88 **
L_4 X T_1	-0.54**	0.39	0.64**	0.62 **	0.31 **	5.84 **	-0.07	-0.05	-0.15 **	1.72 **
L_4 X T_2	0.25**	1.64*	-0.77**	1.74 **	0.33 **	3.98 **	-0.10*	-0.03	-0.23 **	-0.48 **
L_4 X T_3	0.29**	-2.03**	0.14**	-2.35 **	0.02	-9.82 **	0.16 **	0.07	0.38 **	-1.23 **
L_5 X T_1	-1.75**	0.86	-0.31**	-0.61 **	-0.19 **	1.41 **	0.01	-0.03	-0.01	-1.54 **
L_5 X T_2	0.47**	0.91	0.48**	-1.19 **	0.22 **	-0.50 **	0.41 **	-0.06	0.09 **	-0.36 **
L_5 X T_3	1.28**	-1.77*	-0.18**	1.80 **	-0.03	-0.91 **	-0.43 **	0.09	-0.09 **	1.90 **
L_6 X T_1	1.09**	-2.08**	-0.69**	-0.68 **	0.32 **	5.01 **	0.66 **	0.21 **	0.10 **	1.22 **
L_6 X T_2	-0.91**	1.56*	-0.38**	-0.79 **	0.09 **	-2.30 **	-0.46 **	0.19 *	-0.34 **	-1.28 **
L_6 X T_3	-0.18*	0.52	1.07**	1.47 **	-0.41 **	-2.71 **	-0.20 **	-0.41 **	0.24 **	0.06
L_7 X T_1	0.18*	1.02	0.10 **	-1.42 **	-0.02	-4.65 **	-0.20 **	0.07	-0.08 **	0.46 **
L_7 X T_2	0.23*	-0.65	0.05*	-1.16 **	-0.05 *	1.45 **	-0.10 *	0.06	0.45 **	-0.13 *
L_7 X T_3	-0.41**	-0.37	-0.14**	2.57 **	0.06 **	3.20 **	0.30 **	-0.13	-0.37 **	-0.32 **
SE (Hybris)	0.09	0.73	0.023	0.083	0.02	0.08	0.04	0.07	0.01	0.06

* Significant at 5 per cent level ** Significant at 1 per cent level

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RESEARCH ARTICLE

Genetic Diversity in Released and Traditional Rice (*Oryza sativa* L.) Varieties of Tamilnadu using D^2 Statistics

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ABSTRACT

The current investigation was carried out during kharif season of 2021-2022 at Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram adopting randomized block design with the goal to assess the extent of genetic divergence in 33 rice genotypes for eight quantitative characters *viz*., days to 50% flowering, plant height, total number of tillers per plant, number of productive tillers, panicle length, number of grains per panicle, thousand grain weight and grain yield per plant. Analysis of variance disclosed presence of significant variability for yield and yield related characters. Using Mahalanobis D^2 analysis, 33 genotypes were grouped into four clusters. Maximum inter cluster distance was found between Cluster I and III, whereas maximum intra cluster distance was found in Cluster II. Characters such as 1000 grain weight, plant height and days to 50 % flowering contributes significantly to genetic divergence among the other characters studied, signifying extensive genetic diversity and it may be used in rice hybridization programmes for improving grain yield and grain quality.

Keywords: Genetic divergence;traditional rice; Mahalanobis; cluster.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major food crop which plays prominent role in world's economy. It is globally ranked third after wheat and maize in terms of production and it is the staple food crop for nearly two-third of the world's population (Abbade, 2021).It is the major carbohydrate source for barely half of the world's population. Asian countries account for over 95 percent of rice production. In 17 Asian and Pacific countries, 9 North and South American countries, and 8 African countries, it is the predominant dietary energy source. India is one of the world's leading rice producers. Rice cultivation takes up around 43,388,000 hectares of land in India. Rice has a tremendous genetic diversity, with hundreds of variants worldwide, including 6000 types in India. Until 1970, India had over 1,10,000 rice varieties, which were lost as a result of the Green Revolution's insistence on monoculture and hybrid crops(Rathna Priya et al. 2019). Despite white rice is the most often consumed rice, there are several rice varieties that include colour pigments, including black and red rice. Rice variants with coloured pericarps other than white and red were often alluded to as "black rice." Researchers and academics now largely regard black rice as a "super food." The term "super food" refers to foods featuring extraordinarily high nutritional content. Black rice is a high-fibre, antioxidant-rich rice that is intense in vitamins B and E, iron, thiamine, magnesium, niacin, and phosphorus (Saha, 2016). This rice is gaining popularity among rice consumers and dieticians owing to its high nutritional and therapeutic value. For this purpose, the variation of each character is imperative in rice varieties thus, the population's genetic diversity and variability are substantially responsible for the development of new high yielding and high-quality rice varieties that surpass older varieties. Varieties should be produced by a diverse set of parents. Plant breeders employ genetic diversity as a crucial component when selecting the best kind of parents for hybridization programmes. A method leveraging Mahalanobis' D^2 statistics can be used to study the divergence (1936). Based on multivariate analysis, Spark has categorized it into different clusters (1973). The genotypes' degree of genetic diversity is best determined using this technique. The aim of the present study is to analyse the genetic diversity among 33 rice genotypes using Mahalanobis D^2 statistics.

MATERIALS AND METHODS

The genotypes used in this study consisted of 14 popular and 19 traditional varieties of Tamil Nadu collected from two locations – TRRI (Tamil Nadu Rice Research Institute) and Nel Jayaram Institute- Kudavasal. The name and diagnostic features of these varieties are provided in Table 1. Genotypes were raised at plant breeding farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram 608 002. The experiment was laid out in Randomised block design and the genotypes were planted in 3 replications, each replication has 10 rows and in each row 15 plants were planted. Single seedling per hill is maintained. Recommended agronomic practices were followed. Observations on various quantitative characters were taken on single plant basis in five randomly selected plants of genotypes for days to 50 % flowering, plant height, total number of tillers per plant, number of productive tillers per plant, panicle length, number of grains per panicle, 1000 grain weight and grain yield per plant were subjected to statistical analysis and the results were given below. The analysis of genetic divergence was done using Mahalanobis (1936) D^2 Statistics. The genotypes were assigned to distinct clusters, and inter and intra cluster distances, character contribution, as well as mean character performances, were calculated.

RESULTS

The results of analysis of variance unveiled in the Table 2. The variance due to genotypes were highly significant for all the observed character signified those genotypes used in these studies were genetically divergent Table 4. Thirty-three rice genotypes were grouped into four clusters using clustering technique are presented in the Table 3.Cluster I composed of 24 genotypes followed by cluster III composed of four genotypes, cluster II comprised of 3 genotypes, cluster IV composed of two genotypes. The average intra and inter cluster distance is formulated in the Table 4. The

inter cluster distance ranged between 1440.87 and 385.95. The minimum inter cluster distance was observed between cluster between II and IV. The maximum inter cluster distance was observed between I and III. The intra cluster distance ranged from 7.21 to 17.08. Cluster IV had minimum intra cluster value. The relative contribution of individual attribute towards expression of genetic divergence evaluated for each attribute D^2 value is formulated in the Table 5. The top three contributors are 1000 grain weight (57.00 %), plant height (23.67 %), Days to 50% flowering (10.03 %).The other contributors towards the expression of genetic divergence are in percentage is presented in descending order viz., total number of tillers per plant (4.35 %), panicle length (2.65 %), number of productive tillers per plant (1.13 %), grain yield per plant (0.75 %) and number of grains per panicle (0.37 %). Cluster II showed earliest flowering, while genotypes in the Cluster I had dwarf plants in it, Cluster II had maximum total number of tillers per plant, subsequently Cluster III and Cluster II exhibited maximum mean value for the characters viz., number of productive per plant, panicle length, number of grains per panicle, thousand grain weight and grain yield per plant

DISCUSSION

The results of analysis of variance exhibited highly significant difference among the33 genotypes for all characters studied (Table 2). The results obtained in existing study was in agreement with Banumathy *et al.,* (2010), Sudeepthi *et al.,* (2020a), Rathan *et al.,* (2020) and Devi *et al.,* (2020).The studied genotypes were genetically varied and showed a substantial level of variability in all of the examined features, indicating that they were genetically diversified and displayed a large degree of variability in all of the tested characters. The proposed study seeks to assess the magnitude of genetic divergence across 33 rice genotypes and to identify varied genotypes for future research. The Mahalanobis' D^2 statistic grouped 33 rice genotypes into four clusters using the clustering technique (Table 3). Cluster I encompass 24 genotypes, followed by cluster III, which contains four genotypes, cluster II, which comprises three genotypes, and cluster IV includes two genotypes. The results were same as the findings done by Palaniraja and Vennila (2018);Vinod and Dhirendra (2021). The distance between clusters varied between 1440.87 and 385.95 (Table 4). Clusters between II and IV had the shortest inter-cluster distance. The greatest inter-cluster distance was found between Clusters I and III. The distance between clusters ranged from 7.21 to 17.08. Cluster IV is the one with the lowest intra-cluster value. Cluster II had maximum intra cluster value the results were same as findings done by Singh *et al.,* (2020). The relative contribution of individual character towards expression of genetic divergence evaluated for each character (Table 5). The top three contributors are 1000 grain weight (57.00%), plant height (23.67%) and days to 50% flowering (10.03%),Vinod and Dhirendra (2021) findings were same for 1000 grain weight as major contributor, Amudha and Ariharasutharsan (2021) got the similar results for days to 50% flowering as major contributor, Banumathy *et al.,* (2010) studied plant height also a major contributor. The other contributors towards the expression of genetic divergence are in percentage is presented in descending order *viz*., total number of tillers per plant (4.35%), panicle length (2.65%), number of productive tillers per plant (1.13%), grain yield per plant (0.75%) and number of grains per panicle (0.37%). Contribution of different characters to genetic divergence is presented in Fig 1. Yield improvement in any crop through indirect selection depends on selection of traits having direct positive effect on yield. These traits should be selected from the clusters, showing the highest mean values for these characters. Cluster III and Cluster II exhibited highest mean value for the traits *viz*.,number of productive tillers per plant, panicle length, number of grains per panicle, 1000 grain weight and grain yield per plant. Genotypes from clusters III such as Karupu kavuni, Siggappukar, Shivappu kavuni showing desired mean values for yield attributing traits would be chosen for hybridization program to obtain desired seg regants with higher yield.

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Table 1. List of genotypes selected for D^2 analysis

S.No	Genotype code	Name of the genotype	Source of the material
1	G1	CO 5	Tamil Nadu Rice Research Institute, Aduthurai
2	G2	White Ponni	Tamil Nadu Rice Research Institute, Aduthurai
3	G3	ADT 38	Tamil Nadu Rice Research Institute, Aduthurai
4	G4	TRY 2	Tamil Nadu Rice Research Institute, Aduthurai
5	G5	ABT 39	Tamil Nadu Rice Research Institute, Aduthurai
6	G6	CO 52	Tamil Nadu Rice Research Institute, Aduthurai
7	G7	CO 43	Tamil Nadu Rice Research Institute, Aduthurai
8	G8	ADT 46	Tamil Nadu Rice Research Institute, Aduthurai
9	G9	ADT 54	Tamil Nadu Rice Research Institute, Aduthurai
10	G10	TRY 1	Tamil Nadu Rice Research Institute, Aduthurai
11	G11	TRY 3	Tamil Nadu Rice Research Institute, Aduthurai
12	G12	BPT 5204	Tamil Nadu Rice Research Institute, Aduthurai
13	G13	ASD19	Tamil Nadu Rice Research Institute, Aduthurai
14	G14	CR 1009	Tamil Nadu Rice Research Institute, Aduthurai
15	G15	Karupu Kavuni	Nel Jayaram – Paddy Research centre, Kudavasal
16	G16	Salem Sanna	Nel Jayaram – Paddy Research centre, Kudavasal

17	G17	Sivan Samba	Nel Jayaram – Paddy Research centre, Kudavasal
18	G18	Poongar	Nel Jayaram – Paddy Research centre, Kudavasal
19	G19	Soorna Masuri	Nel Jayaram – Paddy Research centre, Kudavasal
20	G20	Seeraga Samba	Nel Jayaram – Paddy Research centre, Kudavasal
21	G21	Kullakar	Nel Jayaram – Paddy Research centre, Kudavasal
22	G22	Aathurkichidi Samba	Nel Jayaram – Paddy Research centre, Kudavasal
23	G23	Sigappukar	Nel Jayaram – Paddy Research centre, Kudavasal
24	G24	Sivapu Kavuni	Nel Jayaram – Paddy Research centre, Kudavasal
25	G25	Kantha Saala	Nel Jayaram – Paddy Research centre, Kudavasal
26	G26	Mottakur	Nel Jayaram – Paddy Research centre, Kudavasal
27	G27	Kalsar	Nel Jayaram – Paddy Research centre, Kudavasal
28	G28	Madumulungi	Nel Jayaram – Paddy Research centre, Kudavasal
29	G29	Sempuli Samba	Nel Jayaram – Paddy Research centre, Kudavasal
30	G30	Kalurundai	Nel Jayaram – Paddy Research centre, Kudavasal
31	G31	Illupai PooSamba	Nel Jayaram – Paddy Research centre, Kudavasal
32	G32	Mapillai Samba	Nel Jayaram – Paddy Research centre, Kudavasal
33	G33	Kaatuyanam	Nel Jayaram – Paddy Research centre, Kudavasal

Table 2. Analysis of variance of 33 genotypes for quantitative characters

source	df	MSS							
		Days to 50% flowering (days)	Plant height(cm)	Total no of tillers per plant	No of productive tillers per plant	Panicle length(cm)	No of grains per panicle	1000 grain weight(g)	Grain yield per plant (g)
Replication	2	348.16	30.13	16.53	0.86	8.41	13.66	0.22	25.72
Genotype	32	1708.39**	3565.20**	35.61**	13.58**	19.61**	1018.26**	67.48**	749.21**
Error	64	27.99	33.29	1.75	1.46	1.39	203.98	0.13	33.58

**significant at 1% level

Table 3. Composition of D^2 clusters of 33 rice genotypes

Cluster	Number of genotypes	Name of the genotypes
I	24	CO5, White Ponni, ADT 38, TRY2, ABT 39, CO 52, CO 43, ADT 46, ADT 54, TRY 1, TRY 3, BPT 5204, ASD 19, CR 1009, Salem Sanna, Sivan Samba, Soorna masuri, Seeraga Samba, Kullakar, Aathur kichadi samba, Kantha Saala, Mottakur, Kalsar, Madumulangi, Illupai Samba
II	3	ADT 46, Poongar, Sempuli Samba
III	4	Karupu kavuni, Siggappukar, Shivappu kavuni
IV	2	Mappillai samba, Kaatuyanam

Table 4. Average intra and inter cluster D^2 and D values of 33 rice genotypes

Cluster	I	II	III	IV
I	**220.42 (14.84)**	461.23 (21.24)	1440.87 (33.77)	439.56 (20.96)
II		**291.86 (17.08)**	630.57 (23.11)	385.95 (19.64)
III			**199.04 (14.10)**	852.31 (29.19)
IV				**52.02 (7.21)**

Table 5: Percentage contribution of different characters towards genetic divergence

SI. No	Characters	Percentage towards genetic divergence (%)
1	Days to 50 % flowering	10.03
2	Plant height	23.67
3	Total number of tillers per plant	4.35
4	Number of productive tillers per plant	1.13
5	Panicle length	2.65
6	Number of grains per panicle	0.37
7	1000 grain weight	57.00
8	Grain yield per plant	0.75

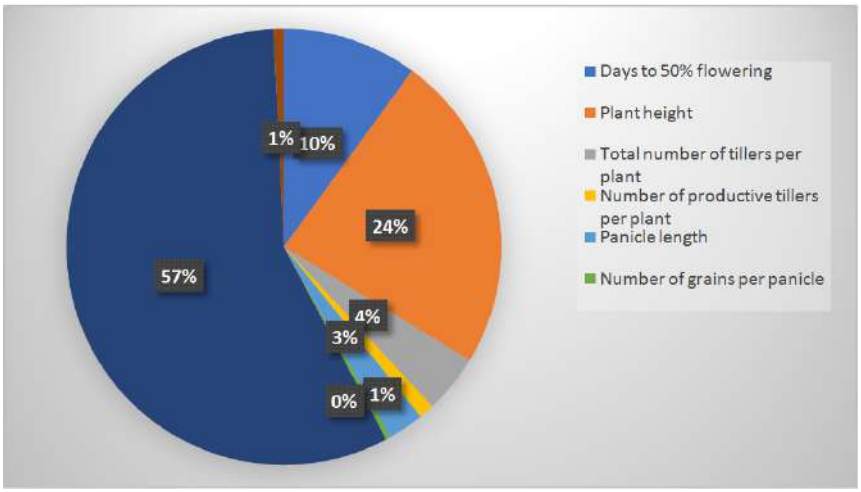

Fig. 1. Percentage contribution of different characters to genetic divergence

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RESEARCH ARTICLE

In-vitro Antioxidant and *In-vivo* Diuretic Potentials of *Abelmoscus esculentus* (L). Monech Leaves Extracts in Rats

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ABSTRACT

The present study was carried out on the *Abelmoscus esculentus* (L). Monech leaves having several pharmacological actions including, diuretic, antibacterial, antioxidant, anti-diabetic activity. In the present study diuretic and *in-vitro* antioxidant activities of ethanol and acetone extract of *Abelmoscus esculentus* (L). Monech leaves was evaluated. Diuretic activity evaluated by measuring various parameters like total urine volume and concentration of different ions i.e.; Sodium, Potassium, Chloride in the urine and *in-vitro* antioxidant activity evaluated by DPPH, Nitric oxide scavenging methods respectively. Ethanolic and acetone leaf extract at dose 500mg/kg showed significant diuretic activity compared with standard drug Furosemide. In antioxidant studies acetone and ethanolic extracts shows the significant activity in DPPH method and acetone extract show excellent activity in nitric oxide method than the standard (Ascorbic acid).

Keywords: *Abelmoscus esculentus* (L). Monech, DPPH, Nitric oxide, Ascorbic acid, Diuretic activity.

INTRODUCTION

Diuretics are drugs that promote the rate of urine flow and sodium excretion. Diuretics alone or in combination with other drugs are used in a variety of clinical situations like hypertension, heart and renal failure, nephritic syndrome and cirrhosis .Diuretics are substances that act within the kidney and promote the loss of fluid from the body [1]. Herbal medicines are considered to be more safe and economical sources of drugs and also contain synergistic and/or side effects neutralizing potential [2]. Therefore, herbal diuretics can be considered as better therapeutic option, because of their relatively safer and milder actions as compared to diuretics used nowadays which produce several adverse effects due to their strong saluretic effects. To be clinically effective, however, such compounds must induce the loss of sodium [2]. This is achieved by compounds interfering with the reabsorption of ions, as well as

water, through the walls of the kidney tubules and this promotes their excretion from the body [3]. Diuretics work by promoting the expulsion of urine and urinary sodium (UNa) from the body and this helps reduce the volume of blood circulating through the cardiovascular system [4]. Anti-oxidants are substances capable to end up free radicals and prevent them from causing cell damage. Free radicals are capable causing a wide number of health problems which include cancer, heart diseases, and gastric problems etc. Antioxidants cause protective effect by neutralizing free radicals, which are toxic byproducts of cell metabolism. Considering all the side effects and great efforts are put to reduce the side effects and to show the effectiveness of present plant. *Abelmoschus esculentus L.* is commonly known as lady's fingers, bhindi, okra or gumbo, is a flowering plant belongs to family Malvaceae. The plant is cultivated in tropical, subtropical and warm temperate regions around the world.[5]. Okra is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds.[6] Okra immature fruits (green seed pods), which are consumed as vegetables, can be used in salads, soups and stews, fresh or dried, fried or boiled.[7]. The entire plant is edible and is used to have several foods [8, 9]. Okra is a popular health food due to its high fiber, vitamin C, and folate content. It is also a good source of calcium and potassium [10]. Greenish-yellow edible okra oil is pressed from okra seeds has a pleasant taste and odor, and is high in unsaturated fats such as oleic acid and linoleic acid [11]. In addition, the plant has been used medicinally in treatment of several disorders[12,13] like Anti-cancer, antimicrobial, hypoglycaemic and anti-ulcer activity[14,15,16]. It is also known for being high in antioxidants. The present work aim was to measure the diuretic and antioxidant activities of the ethanol and acetone extracts of *Abelmoschus esculentus* L. Moench leaves in rats.

EXPERIMENTAL PROCEDURE:

Source of plant material

Abelmoschus esculentus L. Moench leaves was collected from Thirupathi hills, Andhra Pradesh, India in the month of December 2016. It was shown to Prof. Dr. Madhavasetty, Department of Botany, University, Thirupathi, Andhra Pradesh, India to be got identified and authentication. The voucher number is 2135 and the specimen was placed, maintained in our laboratory for further future reference.

Preparation of extract

Abelmoschus esculentus L. Moench leaves was dried in shade and made it into dry powder. Powder was then sieved through the 40 mesh number. Dried powder was subjected to continuous hot extraction procedure in soxhlet apparatus using ethanol and acetone extracts as a solvent. The extracts were evaporated under reduced pressure using rotary evaporator until all the solvent has been removed to get a sample of extract. The residue was dried under vacuum desiccator. The dried extracts were selected for diuretic activity and antioxidant activities.

Determination of total flavonoid content

The amount of total flavonoids was determined using the aluminium chloride ($AlCl_3$) colorimetric assay [17]. 1 ml of sample (1 mg/ml) was mixed with 3 ml of methanol, 0.2 ml of 10% aluminium chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water and remains at room temperature for 30 min. The absorbance of the reaction mixture was measured at 420nm with UV Visible spectrophotometer. Total flavonoid content was calculated on the basis of the calibration curve of gallic acid and expressed in terms of µg/ml.

Experimental animals

Adult male Wistar rats, weighing 150-180g, were procured from the animal house of CES College of pharmacy, Chinnatekur, Kurnool (Reg., no.1278/ac/09/CPCSEA). The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hr light and 12 hr dark day night cycle) and had free access to commercial pellet diet with water ad libitum. The temperature was maintained at 25 ± 10C with relative humidity (50 ±15%). The study was approved by the institutional animal ethical committee (IAEC/CESCOP/2017-14).

Acute toxicity study

The acute toxicity of ethanolic and acetone extracts of *Abelmoschus esculentus* L. Moench leaves was determined as

per The Organization for Economic Cooperation and Development (OECD) guideline no. 423 (Acute Toxic Class Method) [18]. It is observed that the plant extract was not mortal even at the dose of 2000mg/kg. Hence, 250mg/kg and 500mg/kg of this dose were choosen to further study. Acute oral toxicity obtained results were indicated that *Abelmoschus esculentus* L. Moench leaves o f ethanolic and acetone extracts at oral doses up to 2000 mg/kg did not produce any symptoms of acute toxicity and none of the rats died till 72 hours. On observation of rats up to 14 days none of animal died.

F) Diuretic Activity

Diuretic activity was performed by using Lipschitz *et al* [19] method. Healthy rats of either sex were divided in to six groups of six animals each. The group I serve as a control received Normal saline (10 ml/kg.b.wt), group II recieved Furosemide (10mg/kg/ip) as a control with saline. Group III, IV, V and VI were treated with low and high dose (250 mg/kg & 500 mg/kg) of ethanol and acetone extract of Leaves of *Abelmoschus esculentus* (L.) Moench in vehicle respectively. Immediately after the treatment, the animals were placed in metabolic cages (1 animal in one metabolic cage) provided with wire mesh bottom and a funnel to collect the urine. Stainless steel sieves are placed in the funnel to retain fecal matter and to allow the urine to pass. The urine was collected in measuring cylinder up to 4 h for all control and drug treated groups. During this period no food and water was made available to animals. Various parameters like total volume of urine, electrolytes (Na^+, K^+, and Cl^-) were estimated in the urine for assessment of diuretic activity.

Electrolyte Level Analysis

The concentrations of electrolytes (Na^+ and K^+) in urine were determined by using a flame photometer. The concentation of Cl^- ions was estimated by titration with 0.02 N $AgNO_3$ using 5% potassium chromate solutions as indicator.

***In-vitro* antioxidant activity:**

Antioxidant activities of ethanol and acetone extracts were evaluated by two free radical scavenging methods, DPPH assay and Nitric oxide (NO) radical scavenging method.

DPPH Radical Scavenging Assay [20]**:**

The stable DPPH radical was used for determination of free radical scavenging activity of the ethanol and acetone extracts. The 0.1 mM solution of DPPH in methanol was freshly prepared. Different concentrations (20, 40, 80, 120,160 µg/ml) of 3 ml of each extracts were added to 1 ml of methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at values denote the concentration of sample, which is requiring scavenging 50% of DPPH free radicals. Radical scavenging activity was calculated by the following formula.

% scavenging activity was calculated using the formula.

$$I\% = \left[\left(\frac{A_C - A_S}{A_C}\right)\right] \times 100$$

Where Ac**:** Absorbance of control, As**:** Absorbance standard/extract.

The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition on Y-axis and concentration on X-axis.

Nitric oxide radical scavenging activity [21]

The stable nitric oxide radical was used for determination of free radical-scavenging activity of the extracts. At different (20, 40, 80, 120,160 µg/ml) concentrations 4 ml of extracts were added to 1 ml of sodium nitroprusside (25 mM), and incubated at 37ºC for 2 hr. an aliquot (0.5 ml) of the incubation solution was removed and diluted with 0.3 ml Griess reagent (1% sulfanilamide in 5% $H3PO_4$ and 0.1% naphthylethylenediamine dihydrochloride equal

amount), the absorbance was recorded at 517 nm. IC_{50} values denote the concentration of sample, which is requiring scavenging 50% of nitric oxide free radicals. Radical scavenging activity was calculated by the following formula.

$$I\% = \left[\left(\frac{A_C - A_S}{A_C}\right)\right] \times 100$$

Where

A_C**:** Absorbance of control, A_S**:** Absorbance standard/extract.

The effective concentration of sample required to scavenge Nitric oxide radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition on Y-axis and concentration on X-axis.

Statistical analysis

Data were presented as percentage (%) protection and mean ± SEM and were analyzed by one-way ANOVA followed by Dunnett's test for multiple comparisons using Graph pad prism version 5.03. Results were considered significant at p <0.05.

RESULTS AND DISCUSSION

The percentage yield of e ethanolic and acetone extracts of *Abelmoschus esculentus* L. Moench leaves was found to be 22.2 % and 16.4 w/w respectively.

Phytochemical Screening

The ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench was screened for its various phytoconstituents by standard chemical tests. Ethanolic and acetone extracts were found to contain flavonoids, tannins, phenols, steroids and triterpenoids. The results were represented in Table No.1.

Determination of total flavonoid Content of extracts

In the two extracts acetone extract contains high total flavonoid content and ethanolic extract having less flavonoid contents compared to acetone extract. The results were represented Table No.2, Figure No.1.

***In-vitro* antioxidant studies:**

DPPH radical scavenging activity

This assay showed the abilities of the extract and standard ascorbic acid to scavenge DPPH radical at concentration range of 20-160 µg/ml in a concentration dependent manner. Decrease in absorbance with increase in concentration indicates a concentration response relationship in DPPH scavenging activity of extracts. The acetones extract of *Abelmoschus esculentus* L. Moench. Shown significant DPPH scavenging effect with an IC_{50} value of 57µg/ml and the percentage inhibition is 98.02% compared to ascorbic acid. The ethanolic extract showed moderate DPPH radical scavenging activity with an IC_{50} value of 101µg/ml and the percentage inhibition is 90.49%. The results were represented in Table No.3, Figure No. 2.

Nitric oxide radical scavenging activity

The extracts showed a significant nitric oxide scavenging activity between concentration range of 20 to 160 µg/ml in a concentration dependent manner. Both acetone and ethanolic extracts of *Abelmoschus esculentus* L. Moench. Showed good nitric oxide scavenging activity, the (IC_{50}) value of the acetone extract was found to be 15µg/ml and percentage inhibition value is 89.36%. the ethanolic extract showed significant inhibition at 160µg/ml concentration and percentage inhibition value is 85.17% and standard ascorbic acid (IC_{50}) value was 85µg/ml and percentage inhibition value is 67.58%. The results were represented in Table No. 4, Figure No. 3.

Diuretic activity

Effect on urine volume

The effect of ethanol and acetone leaf extracts of Leaves of *Abelmoschus esculentus* (L.) Moench on urine output is shown in table 5. The ethanolic extract (EE) showed a significant diuretic activity by increasing urine output at both 250 and 500 mg/kg concentation respectively (P<0.05 & P<0.001). The extract of acetone (AE) at 500 mg/kg body weight showed significantly (P<0.01) high urine output when compared to the standard group.

Estimation of Urinary Electrolytes

The effect of EEAE and AEAE of Leaves of *Abelmoschus esculentus* (L.) Moench on urinary electrolyte excretion is shown in Table 6. Ethanolic extract Leaves of okra showed a significant increase in excretion of sodium ions. The increased sodium excretion produced by EE was 65.63% and 92.89 % (p<0.001) for the dose of 250 mg/kg and 500 mg/kg respectively, compared to the negative control. Extract of acetone showed an increased in sodiun excretion, this increase was 69.43% and 84.58% at the dose of 250 mg/kg & 500 mg/kg respectively. EE at high dose produced maximum sodium excretion (92.89 %, p<0.001). Enhanced potassium loss was observed for EE at the doses of 250 and 500 mg/kg (16.39% and 24.81%, p<0.001), respectively, compared to the negative control.

CONCLUSION

From the present investigation we can conclude that, both the acetone and ethanolic leaf extracts of *Abelmoschus esculentus* (L.) Moench has shown potent and dose-response diuretic and antioxidant properties, which is significant with acetone extract compared to ethanolic leaf extract of *Abelmoschus esculentus* (L.) Moench. These findings justify at least partly the use of this extract in folk medicine for the treatment of hypertension. Future studies aimed at identifying the active principles accounting for these effects of leaf extract of *Abelmoschus esculentus* (L.) Moench acetone extract may lead to the discovery of a potent diuretic, potentially with antioxidant properties.

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Table No.1: Phytochemical screening of the ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench

S.No	Plant constituents	Inference	
		Ethanol extract	Acetone extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Flavonoids	+	+
4	Phenols	+	+
5	Steroids	-	+
6	Triterpenoids	+	+

Table No.2: Total flavonoid content in ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench

S. No	Extract	Total flavonoid content (Mean±SEM) (GAE µg/g of dry material)
1	Ethanol extract	58.2±0.233
2	Acetone extract	116.25±0.465

Table 03: DPPH radical scavenging activity of ethanol, acetone leaf extracts of *Abelmoschus esculentus* L. Moench.

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC_{50}
1	**Ethanol extract**	20 µg/ml	0.375±0.02	39.79±1.623	101 µg/ml
		40 µg/ml	0.294±0.02	52.83±0.710	
		80 µg/ml	0.136±0.03	78.20±0.510	
		120 µg/ml	0.123±0.01	80.18±1.81	
		160 µg/ml	0.745±0.03	90.49±0.707	
2	**Acetone extract**	20 µg/ml	0.592±0.02	5.12±0.602	57 µg/ml
		40 µg/ml	0.459±0.02	26.33±1.275	
		80 µg/ml	0.189±0.01	69.65±1.062	
		120 µg/ml	0.094±0.01	84.88±1.082	
		160 µg/ml	0.012±0.00	98.02±0.745	
3	**Ascorbic acid**	20 µg/ml	0.050±0.00	91.88±1.816	11 µg/ml
		40 µg/ml	0.020±0.00	96.74±0.775	
		80 µg/ml	0.010±0.00	98.34±1.062	
		120 µg/ml	0.009±0.00	98.55±0.932	
		160 µg/ml	0.006±0.00	98.98±0.752	

Table 4: Nitric oxide radical scavenging activity of ethanol, acetone leaves extracts of *Abelmoschus esculentus* L. Moench.

S. No	Sample	Concentration	Absorbance (Mean±SEM)	Percentage inhibition (Mean±SEM)	IC_{50}
1	**Ethanol extract**	20 µg/ml	0.240±0.03	26.27±0.294	93 µg/ml
		40 µg/ml	0.196±0.02	39.87±0.779	
		80 µg/ml	0.185±0.01	43.04±0.372	
		120 µg/ml	0.116±0.01	64.31±1.532	
		160 µg/ml	0.048±0.00	85.17±0.752	
2	**Acetone extract**	20 µg/ml	0.109±0.01	66.35±0.450	15 µg/ml
		40 µg/ml	0.090±0.01	72.18±0.589	
		80 µg/ml	0.084±0.01	74.23±0.442	
		120 µg/ml	0.072±0.00	77.91±0.372	
		160 µg/ml	0.034±0.00	89.36±0.739	
3	**Ascorbic acid**	20 µg/ml	0.303±0.01	6.95±0.450	85 µg/m
		40 µg/ml	0.176±0.01	45.91±0.226	
		80 µg/ml	0.169±0.01	48.15±0.756	
		120 µg/ml	0.143±0.01	56.13±1.001	
		160 µg/ml	0.105±0.00	67.58±0.388	

Table 5 : Effect of the ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench on urine volume in rats.

Treatment	Dose	Volume of Urine (ml/kg b.w)	% Urinary Excretion	Diuretic Index	Diuretic Action
Saline	10 ml/kg	1.21±0.34	-	-	0.187
Furosemide	10 mg/kg	6.47±1.93***	83.83	5.34	1
EE	250 mg/kg	4.65±2.48*	73.97	3.84	0.71

	500 mg/kg	5.92±1.39***	79.56	4.89	0.91
AE	250 mg/kg	4.57±2.69*	72.79	3.77	0.7
	500 mg/kg	5.13±1.08**	76.41	4.23	0.79

Values are means ± SEM, *n*=6, * < 0.05, ** < 0.01, and***<0.001, significant difference compared to the control.

Table 6: Effect of the ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench On Urinary Electrolyte Excretion in Rats:

Treatment	Dose	Na^+	K^+	Cl^-
Normal saline	10 ml/kg	53.68±3.98	13.14±1.21	62.14±3.65
Furosemide	10 mg/kg	91.69±5.16***	22.64±1.93***	109.82±5.39***
EEAE	250 mg/kg	65.63±7.84	16.39±3.49	78.25±7.59
	500 mg/kg	92.89±4.79***	24.81±2.05***	103.64±8.81***
AEAE	250 mg/kg	69.43±5.81	17.15±2.93	71.53±8.09
	500 mg/kg	84.58±5.03*	19.64±1.98*	94.61±6.48**

Values are means ± SEM, *n*=6, * < 0.05, ** < 0.01, ***<0.001, significant difference compared to the control

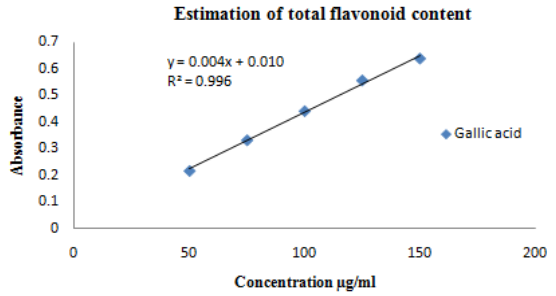

Figure 1: Standard calibration curve of gallic acid for estimation of total flavonoid content

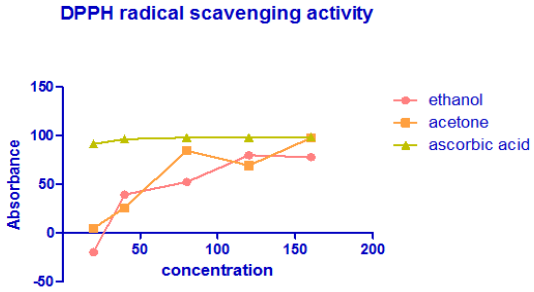

Fig no 2: DDPH radical scavenging activity of ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench. Values are expressed as the Mean±SEM, (n=3)

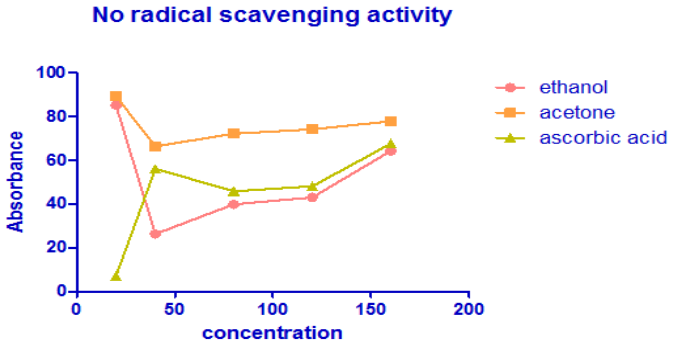

Fig no 3: Nitric oxide radical scavenging activity of ethanol and acetone leaf extracts of *Abelmoschus esculentus* L. Moench. Values are expressed as the Mean±SEM, (n=3)

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RESEARCH ARTICLE

Applications of Fisher's Linear Discriminant Functions for Significant Variables of Finger Ridge Counts of Transwomen

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ABSTRACT

The fingerprint and palmar ridge pattern recognition system recognizes a user by determining the authenticity of a specific anatomical or behavioural characteristic possessed by the user. Protecting sensitive and personal data has become necessary with the ever-increasing integration of computers and the internet into daily life. This paper proposes to assess thesignificant variables of finger ridge counts of Trans women using Fisher's Linear Discriminant Functions. This paper aims to investigate a multimodal biometric identity system using Linear Discriminant Analysis as the backbone of fingerprint recognition and implement such a system in real-time using Wilk's Lambda method.

Keywords : Significant Variables, Finger ridge, Fisher's Linear Discriminant, fingerprint Trans women

INTRODUCTION

The Fisher Linear Discriminant Analysis (also called Linear Discriminant Analysis(LDA) are methods used in statistics, pattern recognition and machine learning to and a linear combination of features which characterizes or distinguishes two or more than one class of objects or facts. The resulting combination of results could be used as a linear classier or, more commonly, for dimensionality reduction before laterclassification.LDA finds an optimal transformation matrix that preserves most of the information, which can be used to discriminate between the different classes. Discriminant function analysis (DFA) is a potential tool that can be used to evaluate the

effectiveness of Indigenous health-and-wellness programs quantitatively. Fisher's linear discriminant function analysis (DFA) is one potential tool that could help in the assessment of the efficacy of health-and-wellness programs. Discriminant function analysis(DFA) is a multivariate statistical technique that uses observed predictor variables to discriminate between two or more groups identified a priori and classify new observations into previously identified groups (Hou and Riley, 2015). The number of discriminant functions is the number of apriori identified groups minus one; thus, for two groups, there would be one discriminant function (Burns and Burns, 2008).

METHODOLOGY

Data Sources and Study Population

The statistically significant variables between groups Fisher's linear discriminant functions were constructed to classify the groups. To analyse the data SPSS (IBM SPSS Statistics for Windows, Version 26.0, Armonk, NY: IBM Corp. Released 2019) is used. Significance level is fixed as 5% (α = 0.05). A total number of 392 participants were enrolled in this study; among them, 31.1% (122) were females, 33.2% (130) were males and the remaining 35.7% (140) were Trans women (Fig.1). For analysis purposes, males and females are combined and constituted 64.3% (252). The fingerprint and palmar ridge samples were collected from the subjects residing in various parts of Tamil Nadu, India. The Canon photo scanner and the Digimizer software were used for sample collection. The fingerprint image is of an 8-bit grey level with a size of 300 × 260 and a resolution of 500 dpi.

Discriminant Function Analysis Variables

Classification method of Fisher's linear discriminant projects higher-dimensional data onto a line and performs classification in this one-dimensional space. The projection maximises the distance between the means of the two classes while minimising the variance within each category. This defines the Fisher criterion, which is maximised over all linear projections, w:

$$J(w) = \frac{|m_1 - m_2|^2}{s_1^2 + s_2^2} \quad \ldots.1$$

Where m represents a mean, s2 represents a variance, and the subscripts denote the two classes. In signal theory, this criterion is also known as the signal-to-interference ratio. Maximising this criterion yields a closed-form solution that involves the inverse of a covariance-like matrix. This method has strong parallels to linear perceptrons. We learn the threshold by optimising a cost function on the training set.The transformation is based on maximising a ratio of between-class and within-class variance" to reduce data variation in the same class and increase the separation between classes. Let us see an example of LDA below (Fig.2).

Multi Classes Problem

Based on the two classes' problems, we can see that the sher's LDA generalises gracefully for multiple classes' problems. Presume we still have a set of D-dimensional samples X = fx(1); x(2); … x(m)g, and there are totally C classes (Fig.3).

RESULTS AND DISCUSSION

Fisher's linear discriminant functions were constructed for statistically significant variables between groups in one-way ANOVA. The results are provided in the following table. Fisher's linear discriminant functions showed that the right "atd" angle correctly predicted a meagre 0.7%Trans women and 99.2% of males/females. The overall prediction was 64.0%. The right "tda″ angle correctly predicted 10.0% of Trans women and 96.4% of males/females. The overall prediction was 65.6%. Right "abcd″ length correctly predicted 98.8% of males/females and only a meagre 0.7% of Trans women. The overall prediction was 63.8% (Table 1).

Fisher's linear discriminant functions results showed that ridge counts in the right middle finger and ring finger correctly predicted 100.0% of males/females and none of the Trans women. The overall prediction by both the finger reading was 64.3% (Table 2).

Fisher's linear discriminant functions results showed that the D2 value in the right hand correctly predicted 95.2% of males/females, and it could predict only 7.9% of Trans women. The overall prediction was 64.0%. D4 values in the right hand correctly predicted 94.4% of males/females and only 9.3% of Trans women. The overall prediction was 64.0% (Table 3).

All significant variables in the right hand

Fisher's linear discriminant function was calculated based on all significant variables measured in the right hand. All variables on the right hand predicted 88.5% of males/females and 32,9% of Trans women. The correct overall prediction was 68.6% (Table 4). The constructed function is given below. Stepwise selection criteria with Wilk's Lambda method were applied to identify and remove the variables that did not contribute much to Fisher's linear discriminant function. The following variables have emerged as significant contributors (Table 5). After using the stepwise procedure to select the important variables, five variables emerged and other significant variables were dropped. The reduced model on the right hand predicted 89.7% of males/females and 32.1% of Trans women. The correct overall prediction was 69.1%, and in the reduced model,the prediction was slightly improved by 0.5% after dropping variables. Fisher's linear discriminant functions results showed that the left "*atd*" angle correctly predicted 99.6% of males/females and only 1.4% of Trans women. The overall prediction was 64.5% (Table 6).

Fisher's linear discriminant functions results showed that ridge counts in the left index finger correctly predicted 100.0% of males/females and only 1.4% of Trans women. The overall prediction was 64.8%. Ridge counts in the left middle finger correctly predicted 100.0% of males/females but it was unable to predict Trans women. The overall prediction was 64.5%. Ridge counts in the left little finger correctly predicted 99.2% of males/females and only 2.1% of Trans women. The overall prediction was 39.5% (Table 7). Fisher's linear discriminant functions results showed that the D2 value in the left hand correctly predicted 96.0% of males/females and only 4.3% of Trans women. The overall prediction was 63.3%. D4 values in the left hand correctly predicted 92.9% of males/females and 9.3% of Trans women. The overall prediction was 63.0%. D2/D4 ratio values in the left hand correctly predicted all (100.0%) of males/females and none of the Trans women. The overall prediction was 64.3% (Table 8). The Fisher's linear discriminant functions showed that total finger ridge counts were correctly predicted for all (100.0%) males/females and only 1.4% of Trans women. The overall prediction was 64.3% (Table 9).

All significant variables in the left hand

Fisher's linear discriminant function was calculated based on all significant variables measured in the left hand. All variables in the left hand predicted 89.3% of males/females and 23.6% of Trans women. The correct overall prediction was 65.8% (Table 10). The constructed function is given below. Stepwise selection criteria with Wilk's Lambda method were applied to identify and remove the variables that did not contribute much to Fisher's linear discriminant function. The following variables emerged as important contributors (Table 11).After using the stepwise procedure to select the important variables, only three variables emerged and the remaining ones were dropped. The reduced model in the left hand predicted 89.3% of males/females and 23.6% of Trans women. The correct overall prediction was 65.8%, and the reduced model predicted as good as the whole model.

All significant variables combined.

Fisher's linear discriminant function was calculated based on all variables measured in both hands, including total finger ridge counts. All the significant variables in the data predicted 89.3% of males/females and 37.9% of Trans women. The correct overall prediction was 70.9% (Table 12). The constructed function is given below.

Stepwise selection criteria with Wilk's Lambda method were applied to identify and remove the variables that did not contribute much to Fisher's linear discriminant function. The following variables emerged as important

contributors.After using the stepwise procedure to select the important variables, only three variables emerged and the remaining thirteen variables were dropped. The reduced model predicted 88.1% of males/females and 25.0% of Trans women. The correct overall prediction was 65.6%, and the loss of overall prediction was 5.3% after dropping thirteen variables (Table 13).

CONCLUSIONS

In this paper, the feasibility of the LDA for Trans women identification was studied. This was accomplished using the Fisher Linear Discriminant (FLD) method applied to the original feature vectors. Also, it is reported that the use of LDA highly decreases the complexity of pattern recognition. However, this method could be used in situations where other methods are not very reliable. In those cases, physiological and genetic tests should be carried out. Such tests could be very time-consuming and expensive and may take much longer than the method presented in this paper. Evaluation of the method on larger data sets will help to study the feasibility of this method in more depth.

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Table 1 Angles and length in Right hand

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Right *atd* angle	M/F : -34.746 + 1.699 × Right *atd* angle T : -32.806 + 1.635 × Right *atd* angle	99.2 0.7	64.0
Right *tda* angle	M/F : -150.795 + 3.691 × Right *tda* angle T : -158.951 + 3.783 × Right *tda* angle	96.4 10.0	65.6
Right *abcd* Length	M/F : -47.924 + 16.890 × Right *abcd* Length T : -51.064 + 17.338 × Right *abcd* Length	98.8 0.7	63.8

Table 2 Finger ridge counts in Right hand

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Right middle finger	M/F : -3.434 + 0.503 × Right Middle Finger Ridge Count T : -4.690 + 0.557 × Right Middle Finger Ridge Count	100.0 0.0	64.3
Right ring finger	M/F : -5.351 + 0.689 × Right Ring Finger Ridge Count T : -6.800 + 0.747 × Right Ring Finger Ridge Count	100.0 0.0	64.3

Table 3 D2, D4 values in Right hand

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
D2 Right hand	M/F : -96.619 + 28.168 × D2R T : -102.316 + 28.907 × D2R	95.2 7.9	64.0
D4 Right hand	M/F : -90.830 + 25.062 × D4R T : -97.053 + 25.832 × D4R	94.4 9.3	64.0

Table 4 All significant variables in the right hand

Group	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Female / Male	= –403.075 + (3.559 × Right '*atd*' angle) + (5.153 × Right '*tda*' angle) + (6.265 × Right '*abcd*' Length) + (0.590 × Right Middle Finger Ridge Count) + (0.765 × Right Ring Finger Ridge Count) + (19.942 × D2 Right hand) + (7.273 × D4 Right hand)	88.5	68.6
Trans women	= –416.753 + (3.503 × Right '*atd*' angle) + (5.238 × Right '*tda*' angle) + (6.924 × Right '*abcd*' Length) + (0.624 × Right Middle Finger Ridge Count) + (0.796 × Right Ring Finger Ridge Count) + (20.002 × D2 Right hand) + (7.712 × D4 Right hand)	32.9	

Table 5 criteria with Wilk's Lambda method

Group	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Female	= –386.065 + (3.427 × Right '*atd*' angle) + (5.138 × Right '*tda*' angle) + (7.963 × Right '*abcd*' Length) + (0.975 × Right Middle Finger Ridge Count) + (21.896 × D4 Right hand)	89.7	69.1
Trans women	= –399.393 + (3.370 × Right '*atd*' angle) + (5.223 × Right '*tda*' angle) + (8.630 × Right '*abcd*' Length) + (1.027 × Right Middle Finger Ridge Count) + (22.379 × D4 Right hand)	32.1	

Table 6 Angles and length in Left hand

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Left *atd* angle	M/F : -30.619 + 1.481 × Left *atd* angle T : -29.182 + 1.431 × Left *atd* angle	99.6 1.4	64.5

Table 7 Finger ridge counts in Left hand

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Left index finger	M/F : -2.186 + 0.324 × Left Index Finger Ridge Count T : -3.277 + 0.368 × Left Index Finger Ridge Count	100.0 1.4	64.8
Left middle finger	M/F : -2.659 + 0.367 × Left Middle Finger Ridge Count T : -3.708 + 0.404 × Left Middle Finger Ridge Count	100.0 0.0	64.3
Left ring finger	M/F : -4.347 + 0.551 × Left Ring Finger Ridge Count T : -5.915 + 0.616 × Left Ring Finger Ridge Count	100.0 0.7	64.5
Left little finger	M/F : -4.746 + 0.688 × Left Little Finger Ridge Count T : -6.242 + 0.757 × Left Little Finger Ridge Count	99.2 2.1	64.5

Table 8 D2, D4 values in Left hand

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
D2 Left hand	M/F : -91.339 + 26.415 × D2L T : -96.354 + 27.051 × D2L	96.0 4.3	63.3
D4 Left hand	M/F : -89.255 + 24.863 × D4L T : -95.691 + 25.668 × D4L	92.9 9.3	63.0
D2/D4 Left hand	M/F : -258.052 + 534.073 × D2/D4L T : -253.923 + 529.161 × D2/D4L	100.0 0.0	64.3

Table 9 Total Finger ridge counts

Variable	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Total ridge count	M/F : -5.948 + 0.084 × Total Finger Ridge Count T : -7.498 + 0.091 × Total Finger Ridge Count	100.0 1.4	64.8

Table 10 All significant variables on the left hand

Group	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Female	= –18212.3 – (0.388 × Left '*atd*' angle) + (0.177 × Left Index Ridge Count) – (0.210 × Left Middle Ridge Count) – (1.791 × Left Ring Finger Ridge Count) + (2.997 × Left Little Finger Ridge Count) – (5304.5 × D2 Left hand) + (5182.1 × D4 Left hand) + (37225.7 × D2/D4 Ratio Left hand)	89.3	65.8

Trans women	= –18205.1 – (0.431 × Left '*atd*' angle) + (0.192 × Left Index Ridge Count) – (0.234 × Left Middle Ridge Count) – (1.721 × Left Ring Finger Ridge Count) + (3.009 × Left Little Finger Ridge Count) – (5302.9 × D2 Left hand) + (5181.4 × D4 Left hand) + (37212.6 × D2/D4 Ratio Left hand)	23.6	

Table 11 criteria with Wilk's Lambda method

Group	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Female	= –132.844 + (1.644 × Left '*atd*' angle) + (0.845 × Left Ring Finger Ridge Count) + (26.015 × D4 Left hand)	89.3	65.8
Trans women	= –138.769 + (1.601 × Left '*atd*' angle) + (0.913 × Left Ring Finger Ridge Count) + (26.846 × D4 Left hand)	23.6	

Table 12 All significant variables combined

Group	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Female	= –20650.3 – (1.816 × Right '*atd*' angle) + (4.408 × Right '*tda*' angle) – (37.507 × Right '*abcd*' Length) + (12.756 × Right Middle Finger Ridge Count) + (0.761 × Right Ring Finger Ridge Count) – (102.5 × D2 Right hand) – (218.8 × D4 Right hand) + (3.901 × Left '*atd*' angle) – (1.140 × Left Index Finger Ridge Count) – (6.554 × Left Middle Finger Ridge Count) – (0.901 × Left Ring Finger Ridge Count) + (3.664 × Left Little Finger Ridge Count) – (5807.5 × D2 Left hand) + (5991.3 × D4 Left hand) + (41949.9 × D2/D4 Ratio Left hand) – (0.496 × Total Finger Ridge Count)	89.3	70.9
Trans women	= –20643.0 – (1.844 × Right '*atd*' angle) + (4.492 × Right '*tda*' angle) – (36.882 × Right '*abcd*' Length) + (12.812 × Right Middle Finger Ridge Count) + (0.789 × Right Ring Finger Ridge Count) – (101.8 × D2 Right hand) – (218.4 × D4 Right hand) + (3.869 × Left '*atd*' angle) – (1.103 × Left Index Finger Ridge Count) – (6.565 × Left Middle Finger Ridge Count) – (0.832 × Left Ring Finger Ridge Count) +	37.9	

	(3.705 × Left Little Finger Ridge Count) – (5805.3 × D2 Left hand) + (5988.7 × D4 Left hand) + (41928.4 × D2/D4 Ratio Left hand) – (0.512 × Total Finger Ridge Count)		

Table 13 Criteria with Wilk's Lambda method

Group	Fisher's linear discriminant function	Correct prediction (%)	
		Group	Overall
Female	= – 224.510 + (3.431 × Right '*tda*' angle) + (0.650 × Left Ring Finger Ridge Count) + (22.318 × D4 Left hand)	88.1	65.6
Trans women	= – 238.534 + (3.513 × Right '*tda*' angle) + (0.719 × Left Ring Finger Ridge Count) + (23.095 × D4 Left hand)	25.0	

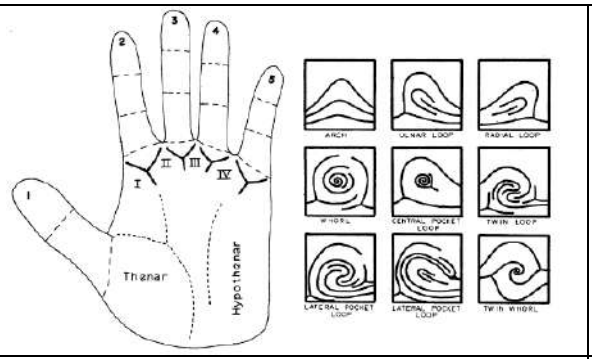

Figure 1. Areas of the hand and various dermatoglyphic patterns

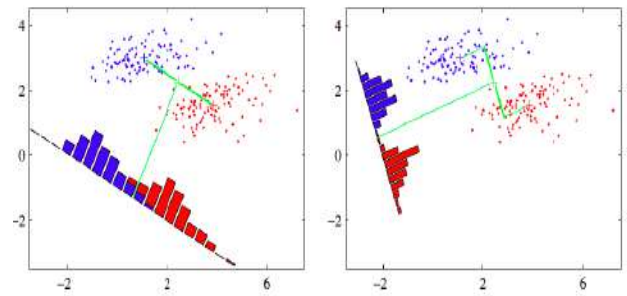

Figure 2. example of Linear Discriminant Analysis (LDA)(after Li and Wang, 2014)

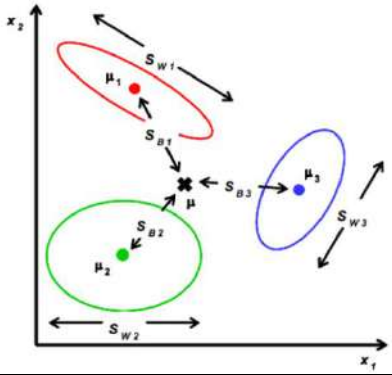

Figure 3. LDA Multi-Class examples(after Li and Wang, 2014)

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RESEARCH ARTICLE

Effect of Phosphorus Enriched Organic Manures on DMP and Soil Health of Cowpea Grown in Vertisols

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ABSTRACT

Cowpea is a food and animal feed crop grown in the semi-arid tropics covering Africa, Asia, Europe, the United States and Central and South America. The grains contain 25% protein and several vitamins and minerals. The plant tolerates drought, performs well in a wide variety of soils and being a legume replenishes low fertility soils when the roots are left to decay. A pot experiment was carried out at the pot culture yard of the Department of SS&AC, Annamalai University, Annamalainagar, Cuddalore District, Tamilnadu. The soil used in this study was black in colour and belongs to Kondal series, Vertisols in order and the taxonomic classification is *Typic Haplusterts*. Recommended doses of N, P_2O_5 and K_2O for cowpea was 25:50:25 kg ha^{-1}. Phosphorus was supplied according to the treatment. Nitrogen and potassium were supplied through urea and MOP fertilizers, respectively. Surface (0-15 cm) soil was collected from an experimental farm, Annamalai University. The soil sample was air dried, powdered and then used for pot culture experiment. The powdered soil was passed through 2 mm sieve and the sieved soil was utilized for the determination of physico - chemical properties adopting the standard procedures. . Application of 100% P_2O_5 enriched pressmud (T_{10}) recorded the highest dry matter production of 39.2 g pot^{-1} against the control (20.3 g pot^{-1}). The bulk density ranged from 1.13 to 1.20 Mg m^{-3}. Higher porosity (44.52%) was recorded in T_{10}. The highest water holding capacity (35.12%) was observed in treatment (T_{10}). The pH of the post – harvest soil of cowpea under the various treatments ranged from 7.49 to 7.90. The highest EC of 1.29 dSm^{-1} was observed in treatment (T_3). Higher organic carbon content (6.5 g kg^{-1}) was recorded in 100% P_2O_5 enriched pressmud (T_{10}) followed by 100% P_2O_5 incubated rock phosphate (T_{13}) (5.7 g kg^{-1}).

Keywords: Cowpea, SSP, Enriched FYM, pressmud, rock phosphate, DMP, soil health

INTRODUCTION

Cowpea is a food and animal feed crop grown in the semi-arid tropics covering Africa, Asia, Europe, the United States, and Central and South America. It originated and was domesticated in Southern Africa and was later moved to East and West Africa and Asia. The grains contain 25% protein and several vitamins and minerals. The plant tolerates drought, performs well in a wide variety of soils and being a legume replenishes low fertility soils when the roots are left to decay. It is grown mainly by small-scale farmers in developing regions where it is often cultivated with other crops as it tolerates shade. It also grows and covers the ground quickly preventing erosion. Organic manures (OM) are a valuable source of nutrients, but their sole application is not sufficient to meet the nutrient requirements of high yielding varieties and often results in poor crop yields [8]. Furthermore, using the generally recommended dose (GRD) of fertilizer is not able to maintain yields vis-à-vis the economic returns of crops, due to fatigue in soil health, and this requires refinement for balanced crop nutrition. Therefore, the sole use of neither OM nor chemical fertilizer can enhance the sustainability of an intensive production system. The use of an appropriate combination of OM and chemical fertilizers [10], depending on soil fertility status, is a step forward for providing balanced fertilization to crops. Such integrated nutrient management (INM) can increase the income of farmers. Application of 100% P2O5 enriched pressmud (T10) recorded the highest dry matter production of 39.2 g pot-1 against the control (20.3 g pot-1). The bulk density ranged from 1.13 to 1.20 Mg m-3. Higher porosity (44.52%) was recorded in T10. The highest water holding capacity (35.12%) was observed in treatment (T10). The pH of the post – harvest soil of cowpea under the various treatments ranged from 7.49 to 7.90. The highest EC of 1.29 dSm-1 was observed in treatment (T3). Higher organic carbon content (6.5 g kg-1) was recorded in 100% P2O5 enriched pressmud (T10) followed by 100% P2O5 incubated rock phosphate (T13) (5.7 g kg-1).

MATERIALS AND METHODS

A pot experiment was carried out at the pot culture yard of the Department of SS&AC, Annamalai University, Annamalainagar, Cuddalore District, Tamilnadu. The soil used in this study was black in colour and belongs to Kondal series, Vertisols in order and the taxonomic classification is *Typic haplusterts*. Cement pots with the size of 45 x 30 x30 cm were used in the study and each pot filled with 40 kg of the processed soil. Cowpea cv. VBN 1 seeds were treated with fungicide (bavistin) carbendazim @ 2g kg^{-1} of seed and after 24 hours, the seeds were treated with *rhizobium* culture (COC 10) + p*hosphobacteria* developed at TNAU using rice kanji as binder. Recommended doses of N, P_2O_5 and K_2O for cowpea was 25:50:25 kg ha^{-1}. Phosphorus was supplied according to the treatment. Nitrogen and potassium were supplied through urea and MOP fertilizers, respectively. Appropriate plant protection measures were taken up against pests and diseases. The crop was grown up to maturity. At maturity the cowpea plant was harvested. Surface (0-15 cm) soil was collected from an experimental farm, Annamalai University. The soil sample was air dried, powdered and then used for pot culture experiment. The powdered soil was passed through 2 mm sieve and the sieved soil was utilized for the determination of physico - chemical properties adopting the standard procedures. The soil is black in colour and belongs to Kondal series, Vertisols in order and the taxonomic classification is *Typic haplusterts*.

RESULT AND DISCUSSION

Dry matter production (DMP) (g pot^{-1})

The data pertaining to dry matter production given table 1. It was found that there was a significant influence was observed with the application of different levels of P_2O_5 enriched organics. Application of 100% P_2O_5 enriched pressmud (T_{10}) recorded the highest dry matter production of 39.2 g pot^{-1} against the control (20.3 g pot^{-1}). Treatments T_{13} and T_7, which received 100% incubated rock phosphate and 100% P_2O_5 enriched FYM, recorded a dry matter production of 36.4 g pot^{-1} and 34.1 g pot^{-1}, respectively. Intermediates T_6 and T_4, which received 75% P_2O_5 enriched pressmud and 100% P_2O_5 (SSP) were found to be statistically on par. This increase in dry matter production

could be due to increased biomass through cell division and multiplication which was influenced by the solubilisation of native phosphorus by different levels P_2O_5 enriched organic fertilizers. These results are in line with the findings of Mukherjee and Rai (2000).

Bulk density (Mg m^{-3})

The bulk density of soil did not differed significantly due to various of P_2O_5 enriched organic manures. The bulk density ranged from 1.13 to 1.20 Mg m^{-3}. Higher bulk density of 1.20 Mg m^{-3} was recorded in the treatment (T_4) which was received 100% P_2O_5 through single super phosphate. However, the bulk density was lower (1.13 Mg m^{-3}) in the treatment (T_{10}) which was received 100% P_2O_5 enriched pressmud. These results are in conformity with the findings of Santhosh (2008), who reported that application of enriched organic fertilizers such as EPM, EFYM and IRP decreased the bulk density while non-enriched inorganic fertilizers alone at different levels increased bulk density of soil. This might be attributed to deterioration of soil structure due to continuous use of inorganic fertilizers (Santhosh, 2008).

Porosity (%)

The data indicated that non significant influence of various treatments on the porosity of soil. Higher porosity (44.52%) was recorded in T_{10} which was received 100% P_2O_5 enriched pressmud. However, lowest porosity was recorded in control (36.63%). Treatment (T_6) (41.95%) did not differ significantly with treatment (T_4) (41. 24%) with respect to per cent porosity. Chalwade *et al.* (2006). They reported that application of a higher level of P - enriched pressmud improved soil aggregation resulting in favourable pore geometry which in turn increased soil porosity.

Water holding capacity (WHC) (%)

The data on water holding capacity revealed that the control treatment (T_1) recorded the lowest water holding capacity (29.18 %). Addition of various treatments increased the water holding capacity from 29.18 to 35.12 %. The highest water holding capacity (35.12%) was observed in treatment (T_{10}), which was received 100% P_2O_5 enriched pressmud. These results are in accordance with the results of Laxminarayana (2006).

Soil pH

The pH of the post – harvest soil of cowpea under the various treatments ranged from 7.49 to 7.90. The highest pH value of 7.90 was recorded in T_{11} followed by T_{12} (7.80) and T_{13}(7.74) and T_5(7.64) and T_6(7.60) and T_7(7.55). The lowest pH value of 7.49 was noticed in the treatment (T_{10}) , which was received 100% P_2O_5 enriched pressmud significant difference was observed between the treatments. This was coupled with release of organic acids during the microbial decomposition of different levels of P_2O_5 enriched organic manures and increased enzymatic activity in soil. These findings endorse the results of Srikanth *et al.* (2000). Application of organic and inorganic fertilizers in combination resulted in decreased soil pH (Chalwade *et al*., 2006).

Electrical conductivity (EC)(dSm^{-1})

The influence of different levels of P_2O_5 enriched organics on electrical conductivity of the post – harvest soil is presented in table 1. The EC values of the post – harvest soil under various treatments varied between 0.51 and 1.29 dSm^{-1}. The highest EC of 1.29 dSm^{-1} was observed in treatment (T_3). The treatment (T_{10}) which received 100% P_2O_5 enriched pressmud registered the lowest EC of 0.51 dSm^{-1} significant difference was observed between the treatments. A similar result was noticed by Yadav *et al*. (2005) due to application of FYM over inorganic fertilizers.

Organic carbon (g kg^{-1})

The data on organic carbon content of the post harvest soil as influenced by different levels of P_2O_5 enriched organic manures are given in table 1. Organic carbon content of the post harvest soil of cowpea differed significantly among the treatments. Higher organic carbon content (6.5 g kg^{-1}) was recorded in 100% P_2O_5 enriched pressmud (T_{10}) followed by 100% P_2O_5 incubated rock phosphate (T_{13}) (5.7 g kg^{-1}). Application of 50 and 100% P_2O_5 enriched farm yard manure recorded the organic carbon content of 5.1, and 5.6 g kg^{-1}, respectively .Lowest organic carbon content was recorded in control (0.47 g kg^{-1}). This could be attributed to addition of enriched pressmud composts and also

due to better root growth and their subsequent decomposition and their influence on the physical – chemical characteristics of the soil. These observations are in conformity with the findings of Babhulkar *et al.* (2000)

Post – harvest Soil available N

The data with respect to the soil available N content as influenced by various levels of P_2O_5 enriched organic manures presented in table 2. Soil available N content differed significantly due to treatmental effects. The available N content of post harvest soil of cowpea in control was (108.41 mg kg^{-1}). Application of 50, 75 and 100% P_2O_5 enriched pressmud registered the soil available nitrogen content of 118.12, 117.94 and 125.85 mg kg^{-1}, respectively. Treatment (T_6) (112.56 mg kg^{-1}) was on par with T_4 (112.41 mg kg^{-1}) which were received 75% P_2O_5 enriched pressmud and 100% P_2O_5 (SSP), respectively. Application of enriched organic fertilizers (EPM, EFYM and IRP) would have lead to greater multiplication of soil microbes, which favours the conversion of organically bound nitrogen to inorganic form. Similar results were reported by several workers (Tolanur and Badanur, 2003 and Rakesh Banwasi and Bajpai, 2006).

Post –harvest soil available P

The available phosphorus content at post harvest soil differed significantly due to various levels of P_2O_5 enriched organic manures and furnished in the table 2. Highest available phosphorus content of 8.71 mg kg^{-1} was recorded in the treatment (T_{10}) which received 100% P_2O_5 enriched pressmud. Followed by T_{13} (8.00 mg kg^{-1}) and T_7 (7.52 mg kg^{-1}) which were received 100% incubated rock phosphate and 100% enriched farm yard manure. The treatment T_6 (5.30 mg kg^{-1}) was on par with T_4 (5.50 mg kg^{-1}) which were received 75% P_2O_5 enriched pressmud and 100 P_2O_5 (SSP). Similarly the treatments T_{12} (6.10mg kg^{-1}) and T_8 (6.63 mg kg^{-1}) were statistically on par. The build up of available phosphorus in enriched fertilizers treated pots might be due to the release of organic acids during microbial decomposition of organic matter, which might have helped in the solubility of native phosphorus thus increasing the available phosphorus content in soil (Santhosh, 2008).

Post - harvest soil available K

The data pertaining to the post harvest soil available K content as influenced by different levels of P_2O_5 enriched organics are given in table 2. It was observed that the post harvest soil available K content differed significantly due to treatmental effects. Highest soil available K content at 147.32 mg kg^{-1} was recorded in the treatment of 100 % EPM (T_{10}) followed 100% IRP (144.60 mg kg^{-1}) and 100% EFYM (144.71 mg kg^{-1}).The available K content did not differed significantly with the treatments T_6 (75% P_2O_5 EPM) (139.58 mg kg^{-1}) and T_4 (100% P_2O_5 SSP) (137.71 mg kg^{-1}). However, the lowest post harvest soil available K was observed in control (T_1) (131.58 mg kg^{-1}). Similar beneficial effects of organic fertilizers on soil available potassium was reported by Siag and Yadav (2004).

CONCLUSION

From the results of present study, it can be concluded that application of 100% P_2O_5 enriched pressmud recorded significantly the highest dry matter production and improved soil health of *Typic Haplusterts.*

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Table 1: Effect of different levels of P_2O_5 enriched organic manures on bulk density (Mg m^{-3}), pore space (%) and maximum water holding capacity (WHC) (%) in the post – harvest soil of cowpea cv. VBN 1.

Treatments	DMP (g pot^{-1})	Bulk density (Mg m^{-3})	Pore space (%)	Water holding capacity (%)	pH	EC (dSm^{-1})	Organic carbon (g kg^{-1})
T_1 – Control (No P_2O_5)	20.3	1.17	36.63	29.18	7.81	0.76	4.7
T_2 – 50 % P_2O_5 (SSP)	22.1	1.15	37.10	29.52	7.75	1.01	4.9
T_3 – 75 % P_2O_5 (SSP)	26.9	1.15	38.91	30.92	7.63	1.29	4.9
T_4 – 100 % P_2O_5 (SSP)	31.0	1.11	41.24	32.20	7.64	0.83	5.0
T_5 – 50 % P_2O_5 (EFYM)	23.9	1.15	37.76	30.08	7.64	0.64	5.1
T_6 – 75 % P_2O_5 (EFYM)	31.3	1.20	41.95	32.65	7.60	0.61	5.1
T_7 – 100 % P_2O_5 (EFYM)	34.1	1.20	43.07	33.60	7.55	0.58	5.6
T_8 – 50 % P_2O_5 (EPM)	28.9	1.13	40.75	31.63	7.60	0.66	5.4
T_9 - 75 % P_2O_5 (EPM)	32.1	1.13	42.66	32.94	7.59	0.58	5.9
T_{10} – 100 % P_2O_5 (EPM)	39.2	1.13	44.52	35.12	7.49	0.51	6.5
T_{11} - 50 % P_2O_5 (IRP)	26.5	1.20	38.25	30.49	7.90	0.66	5.1
T_{12}- 75 % P_2O_5 (IRP)	29.3	1.17	40.98	31.94	7.80	0.73	5.2
T_{13} - 100 % P_2O_5 (IRP)	36.4	1.17	43.12	34.15	7.74	0.96	5.7
SEd	0.69	0.04	0.96	0.65	0.06	0.08	0.07
CD(p=0.05)	1.50	NS	NS	NS	0.14	0.16	0.14

Table 2: Effect of different levels of P_2O_5 enriched organic manures in post – harvest soil available nitrogen, phosphorus and potassium (mg kg^{-1}) of cowpea cv. VBN 1.

Treatments	Available Nitrogen	Available Phosphorus	Available Potassium
T_1 – Control (No P_2O_5)	108.41	4.14	131.58
T_2 – 50 % P_2O_5 (SSP)	109.83	4.56	133.32
T_3 – 75 % P_2O_5 (SSP)	110.40	5.21	137.06
T_4 – 100 % P_2O_5 (SSP)	112.41	5.50	137.71
T_5 – 50 % P_2O_5 (EFYM)	110.35	4.65	135.06
T_6 – 75 % P_2O_5 (EFYM)	112.56	5.30	139.58
T_7 – 100 % P_2O_5 (EFYM)	120.89	7.52	144.71
T_8 – 50 % P_2O_5 (EPM)	118.12	6.63	144.56
T_9 - 75 % P_2O_5 (EPM)	117.94	7.05	145.03
T_{10} – 100 % P_2O_5 (EPM)	125.85	8.71	147.32
T_{11} - 50 % P_2O_5 (IRP)	111.42	4.63	134.95
T_{12}- 75 % P_2O_5 (IRP)	115.93	6.10	142.04
T_{13} - 100 % P_2O_5 (IRP)	122.26	8.00	144.60
SEd	1.48	0.13	1.02
CD(p=0.05)	2.11	0.27	2.21

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RESEARCH ARTICLE

Physiological / Anthropometrical Aspects of Volleyball Players with their Analytical Assessment

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ABSTRACT

Recent research in the domain of team sports training has been built the fitness profile for each single sport. However, generally the team sports training requires a multifaceted strategy to acquire the knowledge of all the performance aspects that influences the entire game. The progress in fitness levels also required to attain the superior outcomes. Every player from the team as the disciplines consist the definite physiological as well as anthropometric profile because of certain aspects and requirements for each game position. Although, few mutual features can be defined while comparing various sports. The accurate meaning of reference profiles is necessary to perform the proficient talent selection processes and significant for appropriate training of elite populations. Researchers and experts have performed many studies in the domestic as well as foreign nations on anthropometric, physiological variables and physical fitness of Volleyball. Nevertheless, the Indian players especially from the universities still need the comprehensive research on the physical fitness anthropometric as well as physiological variables for volleyball players in order to provide a top-ranked team to the nation. 2) In the proposed study, there is the comparison of physical, anthropometric and physiological variables among north zone and west zone Indian university volleyball players.

Keywords: Volleyball, Anthropometric, Physiological, Physical Education, Fitness Effectiveness, Sports

INTRODUCTION

Physical Education (PE) is the most significant element of ability-oriented training. As India has the great prominence upon the formation of New Curriculum Reform (NCR) as well as ability-oriented learning of PE, the PE status shows a lot of significance in core education. One of the key exercise is volleyball in PE, not only due to its transferring role in the tradition of certain sport, but also it has the extraordinary efficient features that would be

highlighted in this dissertation. PE has the formation of NCR showing the brand-new prototype of the overall PE condition that would consist some re-consideration to PE program arrangements in schools. In between several sports items, researchers have made some in-depth examination into those that involve exclusive feature efficiency and make utilize of them effective. Anxo-action operation is important that defines the appropriate arrangement of PE layout within the NCR framework. This dissertation performs the research, depending on the significance of volleyball discussed above, on the usage of volleyball's characteristics efficiently in the physical progress of primary school students as well as its exertion channel. Researchers explore the volleyball sports and provide the rational conclusion with some efficient theoretical supervision to the PE arrangement in NCR. Sport is now no longer is just a passion or recreation but often considered as a science (Nesti, 2016). Any sport can be assessed in several aspects of the activity pursuing with a scientific temper. It involves the estimation of the overall consequences of a match with scoring points/goals, the player's positions and several other intricacies/levels (Basterfield et al., 2016). Volleyball can be enjoyable by people of all ages with different skill levels in both outdoor/indoor situations. It demands a high level of physical fitness as it helps in burning the 585 calories within the 45 minutes of game (Eskici, 2016). Volleyball progresses the tone and muscle strength, using the lower legs, abdomen, thighs, shoulders, arms and upper body (Colakoglu et al., 2017). Furthermore, Volleyball also enhances the reflexes, balance and hand-eye coordination among the Players (Agopyan et al., 2018). It has the potential to indulge the players in an active communication and teamwork, as it is a team sport. Volleyball consists six rotational court positions as Middle back, Left back, Middle front, Left front, Right back and Right front (Van Haaren et al. 2014) with respect to the players. Further, it has the two zones as the defense as well as attack. The game changes according to the shifting of every player's positions depending on the game strategy (Amar and Otman, 2018) with respect to the other players. There are 6 players on court at any given time of the game. Out of them, initially there is one setter (S) and five attackers (A). With the progress of game, one libero (L) can substitute one of the attackers. The nature of the game does not affect the physiological and physical aspect of every player characterization (Hu et al., 2016). Therefore, it becomes nearly impossible to determine the playing position that depends on any one of these aspect.

These conditions begin the progress of new strategies in order to categorize the players into their playing positions depending on several factors (Fransen et al., 2016). Numerous scientific and technical tools play significant role in such procedure like machine learning technique, various software and measuring kits. Generally, several variables for motor fitness and anthropometric are utilized to analyze the performance level of a player as well as can be utilized in player's quantification in a specific game (Opstoel et al, 2015). The consequences are generally utilized as the supportive aspects to estimate the player's quality. Such information are appropriate for the real-time applications (Silva et al., 2016). After the collection of data, the preparation strategies can be utilized to optimize the performance. Traditional strategies involve the data transformation as well as data pre-processing methodologies. Statistical assessment outlines the data variations in context with several performance traits (Kavanaugh et al., 2018). The current research used two categorization systems as XG Boost and Support Vector Machine (SVM). Extreme Gradient Boost (XG Boost) uses the Model based learning technique and SVM needed the tuned hyper-parameter learning system. Most of the research involves the Grid search method to regulate the hyper-parameters, as it is the most efficient SVM fit (Bui et al., 2016). On the other hand, XG Boost represents the gradient boosting algorithm that is a systematic Gradient Boosting Machine (GBM) model utilized to prevent over-fitting and resulting as the delivery of exponentially superior performance.

Historical Development of Volleyball

Globally, millions of people are the huge fan of Volleyball. Several nations consider this sport as the top-level competitive game. Everyone accepts this sport wholeheartedly due to its attractive features. Volleyball is a thrilling game with the providence of great physical fitness. It needs a small area with lesser resources. Volleyball is a sport that not only engaging the hands in spiking or receiving the ball, but also engaging the entire mind as well as body in this sport. As far as the accuracy and action concerned, volleyball is possibly the prominent balling sport in the world. This game is very exciting as anything can happen at any time despite of result. Volleyball has a unit of six players that make it as a team game instead of machine sport. Volleyball has consistently progress from its commencement until the current day. William Morgan was structured as well as conceived this game. In 1895 at

Holyoke in Massachusetts, USA, he was working as the Director of Physical Education at the Young Men's Christian Association. Morgan was looking for a recreational activity for several years that could be psychologically as well as physically refreshing and must be appropriate for each kind of age group. At the beginning, Morgan tried his hand in Tennis, although the issue is buying the new material and rackets that open the way for the discovery of Volleyball. Nevertheless, at the beginning, a height of six feet was achieved by using the Tennis net, above that the Basketball bladder was volleyed. Since the flight over the net was slow and the bladder was too light, Morgan utilized the Basketball. However, it was too heavy as well as large to volley above the net. Further, Morgan proposed some guidelines to the manufacture that include the developing a ball that was neither big nor heavy used as Basketball. Such kind of certain specifications was given to was given to Spalding and brothers. Afterwards, a ball was manufactured i.e. lighter and smaller as used in Basketball and then work on the approval of the net. In 1896, the first game of Volleyball was played in front of Physical Directors at Springfield College at a conference. It was played with a lot of interests and first named as Mintar. After that Morgan changed the name as Mintorna the. Alfred T. Halstead proposed the name as 'Volleyball' after the careful assessment of the sport and witnessing the nature of the game being played that was unanimously accepted.

Young Men's Christian Association, Physical Director's Society further made the made the rapid development in this sport under their supervision. This sport quickly moved out from its boundaries of homeland (USA) within a short timed. USA was the first nation that play this sport in 1900 and Cuba was the subsequent immediate nation to play the Volleyball in 1905. It was one of the trending army game at the time of World War-1 and US Soldiers carried this sport in several regions of the world. It is significant to make and study the guidelines accurately before launching a game globally. Therefore, a Special Committee of Young Men Christian Association performed this task efficiently in 1912. American Sports Company at New York published an entire Volleyball guide in 1917. In the beginning of 20th Century, Y.M.C.A. introduced the Volleyball in India. Y.M.C.A. College of Physical Education at Madras was one of the first institutions to take up Volleyball. This institute also provided the training to the physical education instructors who have taken this sport to almost entire regions of the Indian sub-continent. Nowadays, Volleyball is the popular sport played at the entire breadth and length of the nation in the rural areas, villages, public playground, colleges and schools. Day-by-day, the popularity of this sport is increasing at all the regions of India. Throughout the year, the conduction of coaching camps, competitions and tournaments in great number are the clear sign that Volleyball has taken deep root on Indian soil. It becomes one of the championship sport for inter-collegiate as well as inter-school competitions in several regions and the inter-university championships held every year. In 1950, a controlling body was set up as the Volleyball Federation of India, which coordinates the activities of member associations as well as endorses this sport at the national level. Several states conduct the national championships as well as organize annual state championships for girls, boys, women and men every year that becomes the highpoint of Indian Volleyball. In India, the beginning of national championships in Volleyball was from the years, 1952 for Men, 1953 for Women, 1956 for junior boys and 1975 for junior girls. Furthermore, Federation Volleyball Tournament for women/men as the new event was launched in 1979. All India Inter-Zonal Sub-senior Volleyball Tournament was initiated in 1980 within the age group of 18-22 years. Apart from that, other agencies as the sports clubs performed numerous open as well as local championships. Volleyball championships are the most trending one in sub-urban regions and few of that regions consider this sport as the character of youth festivals.

Volleyball sportsperson also get the privilege to go overseas. In 1952, the foremost Indian team in the World Volleyball Championship chosen at Calcutta participated held at Moscow for male participants as well as it was India's first participation in the International field. At that Championship, the Indian team could only get the 8th position. Afterwards, the Indian team visited China in 1954 and took participation in a series of competitive games against local teams. In 1955, India had the distinction of winning the Asian title at the first Asian Volleyball Championship. U.S.S.R. sent its one of the leading Volleyball clubs toured India in 1955 and participated in exhibition tournaments at several centers. Meanwhile, Indian team has performed well in numerous international events, thus gaining its experience by competing with highly ranked world teams. Furthermore, there is the implementation of coaching and training schemes in the supervision of Indian and foreign skilled coaches. In India,

such championships have significantly helped the volleyball players to give up on the traditional procedures and implement highly advanced techniques.

RESEARCH METHODOLOGY

As the research methodology proposes a systematic methodology to investigate the subject from the beginning of problem identification to the final conclusion. The primary objective to design an optimized methodology was to conduct the research in a valid and scientific way. This paper focuses on to elaborate the procedures adopted that involves choosing of participants, attributes selection, design of research methodology, pilot study, data reliability, instrumental validity, tester's and subjects validity, subject orientation, data collection, statistical procedures and test administration.

Participant Selection

This study has the purpose to assess the playing capability of volleyball players from the certain motor fitness as well as anthropometrical attributes amongst all India and Inter University level athletes. Primarily, 120 students from All India level players and 120 students from various colleges in North India states were selected/tested in order to attain the objectives. The volleyball players have the age group of 17-25 years and monitor the performances of 240 volleyball players. Participants had at least three years of prior playing experience in volleyball at all India and Inter University levels.

Attributes Selection

The attributes as anthropometrical, physical and physiological factor play the significant roles in overall performance. The experts perform the comprehensive study by researching the several journals, coaching manuals, unpublished theses, e-resources and books. They investigated on the correlation of standard skills with selected anthropometrical, physical and physiological fitness attributes. This study has the in depth research on several independent attributes depending on the comprehensive literature review. The independent variables are

a) **Anthropometrical** – Body Weight
b) **Breadth Measurements** – It has Femur Breadth and Humerus Breadth
c) **Length Measurements** – It has Palm Length, Hand Breath, Hand Length, Leg Length, Arm Span, Arm Length and Standing Height
d) **Girth Measurements** – It has Thigh and Calf, Hip, Waist, Chest, Arm Girth Flexed and Arm Girth Relaxed
e) **Motor Fitness Variables namely** – Reaction Time, Co-ordination, Balance, Power, Agility and Speed.

While, dependent variable refers the playing capability. It represents the performance factor that was individually analyzed by professionally qualified volleyball expertise. A pilot study was performed to validate the research methodology prior to the formal research, to estimate the viability as well as time constrain of this work. For this, twenty five volleyball players were chosen without any participation in this research from Manipal University Jaipur, Rajasthan, India based on selected criterion attributes by the examiner. According to the performance capability and responses of volleyball players at the time of pilot study, this model was developed. The pilot study has the outcomes to validate the feasibility of suggested research model. Although, the primary matter of concern was low participation rate. Such pilot study aimed to collect the information about 25 youth players of volleyball to perform anthropometric, physical and physiological tests, including total twenty-two tests. Procedures to lessen the number of tests within shorten time is essential and still explored for improving the participation rate.

COLLECTION OF DATA

This section describes the details of collecting the data from All-India and Inter-state level of volleyball players on chosen anthropometric, physical and physiological fitness attributes. The purpose of this research work has been fulfilled by taking the consent from team members, tournament committee directors and coaches. The administration

times/dates were decided according to receiving consent. Subsequent preventive measures were considered to assure the safety of every coach and participants:

a) During the medical examination, review of fitness certificates as well as medical history of the participants generated.
b) Participants checked and signed the informed permission letter.
c) Investigator fully described the testing processes prior to testing.
d) The investigator or the participant can dismiss the test at any time of experiment.
e) Testing site displays the emergency numbers.
f) Biohazard precautions involved by wearing the latex gloves as the testing personnel object and changed for every participant.
g) A confidential file can carry the complete data and materials relating with every participant in a locked and coded form.
h) Proper disposal of biohazard material after the test completion to attain the approval from athletes.

RESULT AND DISCUSSION

The existing research work involves forty independent attributes and one dependent attribute, referred as playing potential of volleyball athletes. Data collection was subjected to conduct the statistical assessment as described in upcoming sections. Pearson's product-moment correlation coefficients can compute to find the correlation between chosen motor fitness attributes and anthropometrical variables with the volleyball playing potential of the players. This study also involves the analysis of playing position of the players such as Blockers, Liberos, Setters and Spikers with their respective sample size as N = 66, N = 43, N = 46 and N = 85. The testing process of hypothesis with respect to the outcomes achieved related with the confidence level. Such examination was often known as the assessment of significance subsequently it examines whether the correlation among the predictor variable and criterion were substantial or not. This study involves the assessment of data on SPSS software by using the t-test on this sample size that involves several attributes and made comparison in between All-India Inter-University as well as Inter-University volleyball players. It also mentions the value of mean and standard deviation for all the considered anthropometric and motor fitness attributes.

The potential of volleyball players at All-India Inter-University level and Inter-university level were estimated from the chosen motor fitness attributes and anthropometrical variables. The playing potential of volleyball were estimated with the help of predictor variables a selected criterion attributes. Multiple regression approaches as stepwise selection was utilized to estimate the equation for prediction level. Confidence level was set as 0.05 to significance test in all cases that which was appropriately considered. This research work obtained the 't' and F value, if that were higher than the standard values, then the null hypothesis were precluded to the influence involving the existing substantial correlation among dependent and independent attributes. In addition, if the attained values were lower than the requisite one at 0.05 level, then there will be the acceptance of null hypothesis with the influence of existed no substantial correlation between the means attributes under research work. The descriptive statistics on certain motor fitness attributes as well as anthropometrical variables of the participants are depicted in the subsequent tables. Table 3 depicted the descriptive statistics in terms of Standard deviation, Mean, Maximum, Minimum and Range of motor fitness attributes and anthropometrical variables with the playing performance of Inter-college level Volleyball Players. The graphical representation shows in two parts due to the complexity of data in Fig. 4.1 and Fig. 4.2.

This research work has the objective to search and assess the certain constituents of physiological variables, anthropometric measurements and physical fitness among the All-India Inter-University level and Inter-university level volleyball players. In addition, this work also presents the data analysis of volleyball players according to their respective playing position with the specific attributes to measure motor fitness level and anthropometric variables. The findings reveal a substantial difference in several constituents of physical fitness among the volleyball players of

All-India Inter-University level and Inter-university level. A substantial difference was monitored among observed between All-India Inter-University level and Inter-university level volleyball players in terms of agility. The outcomes of greater coordination, balance, power, speed and agility in All-India Inter-University level volleyball players may reveal in their competition intensity as well as higher training at the great performance level. Conversely, there is no difference of reaction time among All-India Inter-University level and Inter-university level volleyball players that was indirectly enumerated by Nelson hand reaction time examination.

CONCLUSIONS

Anthropometric characteristics of the players refer as the significant precondition for the accomplishment of the availability in same sport, influencing the performance of the player as well as are essential for achieving the best performance of volleyball skills. The motor ability represents the general athletic potential of the players. In volleyball, several teams play against with each other by maintaining the ball above the head. In such kind of sport, height is the most significant physical characteristic. In volleyball, individual physical performance, anthropometric traits, tactical skills and technical knowledge are the most substantial attributes that contribute in the team success during the international or national level competitions. The volleyball performance has the advantage in terms of optimal physique. Only if a volleyball team has fully loaded with the complete necessary anthropometric traits can surly win the game with great performance. Depending on the outcomes of this research work, the subsequent conclusions have been made:

a) The All-India Inter-University level volleyball players had substantially higher level of coordination, balance, power, speed and agility with respect to Inter-University volleyball athletes.
b) The All-India Inter-University level volleyball players were substantially heavier as well as taller with respect to Inter-University volleyball athletes.
c) The All-India Inter-University volleyball players had substantially larger leg length with respect to Inter-University volleyball athletes. Conversely, the Inter-University volleyball athletes had substantially larger forearm length.
d) The All-India Inter-University volleyball players were estimated to consist substantially larger biacromial diameters, bicondylar humerus, thigh circumferences, upper arm and forearm with respect to Inter-University volleyball athletes. On the other hand, Inter-University volleyball athletes have substantially larger hip diameter.
e) The All-India Inter-University volleyball players had substantially lesser percentage body fat, subscapular skinfold thicknesses and biceps with respect to Inter-University volleyball athletes.
f) All-India Inter-University level volleyball players had substantially greater lean body mass and body density.
g) All-India Inter-University level volleyball players had substantially higher level of mesomorphy, while, Inter-University volleyball players had the higher level of Endomorphy.
h) All-India Inter-University level volleyball players had substantially higher mean values obtained for the entire physiological attributes with respect to Inter-University volleyball athletes.
i) A substantial difference is present between the different playing positions of volleyball athletes for all the anthropometric variable computation excluding the hip diameter, forearm circumference and BMI.
j) There were the absence of substantial differences between the different playing positions of volleyball athletes for all the percentage body fat, body density and skinfold thicknesses. Conversely, lean body mass as well as total body fat were different according to the playing positions of the athletes.
k) Power and speed were substantially varied according to the playing positions of volleyball athletes.
l) Expiratory/inspiratory reserve volume have shown substantial differences between the playing positions of volleyball athletes.

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Table 1. Anthropometric, Physical and Physiological attributes with their respective measurement and testing tools and SI unit

S. No.	Variables	Measurement/Testing tools	SI Units
1	Body weight	Electronic Weighing Machine	In Kilograms
Breadth Measurements			
2	Femur breadth	Lufkin Anthropometric Tape	In Centimeters
3	Humerus breadth		
Length Measurements			
4	Height	Stadiometer	In Centimeters
5	Leg length	Lufkin Anthropometric Tape	
6	Arm span		
7	Arm Length		
8	Palm length	Small Sliding Caliper	
9	Hand breadth		
10	Hand length		

Girth Measurements			
11	Calf	Lufkin Anthropometric Tape	In Centimeters
12	Thigh		
13	Hip		
14	Waist		
15	Chest		
16	Arm girth flexed		
17	Arm girth relaxed		

Table 2. Motor Fitness variables

S. No.	Attributes	Measuring Tool	SI Units
1	Reaction Time	Chronoscope	In Seconds
2	Co-ordination	Alternate Hand Ball Toss Test	In Numbers
3	Balance	Stork Stand	In Seconds
4	Leg explosive power	Standing broad jump	In Meters
5	Agility	T Agility Run	In Seconds
6	Speed	50 Meters run	In Seconds

Table 3: depicted the descriptive statistics in terms of Standard deviation

Variables	Range	Minimum	Maximum	Mean	SD (±)
Body Weight	20.28	58.46	78.76	70.07	8.48
Height	22.55	160.63	182.98	180.91	6.42
Arm Length	16.47	70.32	86.87	81.28	4.91
Arm Span	34.56	158.89	193.95	171.98	10.45
Leg Length	28.98	89.95	118.86	100.12	5.39
Hand Length	3.45	18.34	21.89	20.18	0.90
Hand Breadth	1.83	5.76	7.57	6.61	0.53
Palm Length	2.02	9.78	11.87	10.72	0.54
Humerus Breadth	1.93	5.65	7.61	6.54	0.62
Femur Breadth	2.13	7.92	10.07	9.01	0.64
Arm Girth Relaxed	8.92	20.90	30.94	25.93	2.58
Arm Girth Flexed	10.28	24.17	34.46	29.41	3.57
Chest	22.29	76.13	98.32	87.72	9.32
Waist	27.93	64.58	92.61	79.68	7.47
Hip	24.32	75.12	99.38	87.47	7.86
Thigh	38.59	43.42	81.97	63.86	12.98
Calf	9.25	30.17	39.28	33.64	2.84
Speed	0.53	7.49	8.08	7.87	0.78
Agility	1.34	10.81	11.97	19.38	1.52
Leg Explosive Power	0.31	1.72	1.96	1.87	0.08
Balance	3.78	7.08	10.89	17.47	8.63
Co-ordination	7.97	25.97	33.96	18.69	1.67
Reaction Time	0.16	0.25	0.42	0.117	0.014

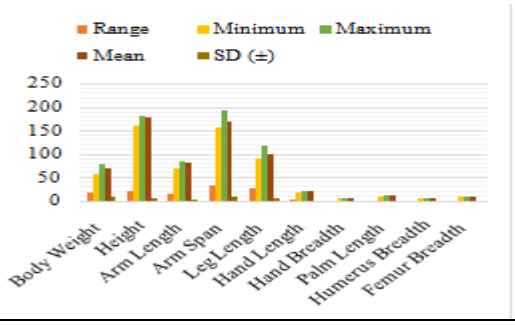

Fig. 1 Graphical representation of certain motor fitness attributes and anthropometric variables (Part-1)

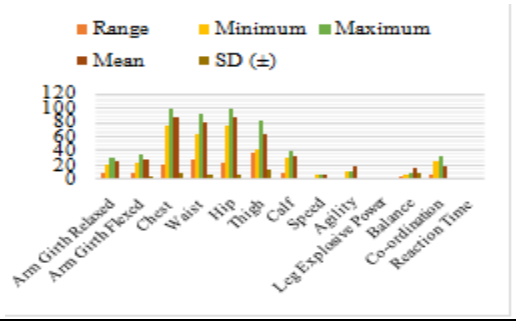

Fig. 2 Graphical representation of certain motor fitness attributes and anthropometric variables (Part-2)

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RESEARCH ARTICLE

Variability and Association Studies in Varieties and Traditional Rice (*Oryza sativa* L.) of Tamil Nadu

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ABSTRACT

The current study was conducted to investigate the variability in thirty-three (33) rice germplasm lines for quantitative characteristics. ANOVA revealed extremely significant differences for all eight traits, indicating the presence of genetic variability across the genotypes tested. High PCV and GCV were recorded for traits such as grain yield per plant (39.83 percent), plant height (34.00 percent), 1000 grain weight (22.59 percent), and days to 50% flowering (21.77 percent), indicating a smaller environmental influence on trait expression, implying that genetic improvement through direct selection is feasible for the evaluated traits. High heritability combined with genetic advancement indicates the influence of additive gene action, and selection would be effective in such traits as 1000 grain weight (99.38 percent), plant height (97.24 percent), days to 50% flowering (95.24 percent), grain yield (87.65 percent), total number of tillers per plant (86.55 percent), and productive tillers per plant (73.42 percent). Plant height, number of productive tillers per plant, number of grains per panicle, and thousand grain weight all correlated positively with grain yield per plant. Grain yield per plant was directly affected by traits such as 1000 grain weight and the number of productive tillers per plant.

Keywords: Biometric, Variability, traditional rice, Correlation.

INTRODUCTION

For more than half of the world's population, rice (*Oryza sativa* L.) serves as their primary source of sustenance. In Asia, 90% of the world's rice is produced and consumed, where 50% of the world's population lives Virtually 43% of the grains produced for nourishment in India are rice (Shivani *et al.,* 2021). Rice that has been polished (or milled)

seems to have the embryo and bran layers removed during processing, having left largely endosperm behind. Most consumers are concerned that rice endosperm is deficient in numerous vitamins and phytonutrients. According to Ritchie *et al*. (2018), micronutrient deficiencies, particularly in children and pregnant women, are prevalent in the Indian population. As a result, finding the genotypes that were significant in micronutrients especially zinc and Iron content along with yield trait is crucial for increasing productivity and rice production. The development of high yielding cultivars is necessary due to the traditional rice cultivars' low yield potential. According to estimates, there will be a 60 percent increase in the consumption of rice by 2050 to accommodate the 9.7 billion people who will dwell the globe (Wani *et al*., 2020). For increasing demand, the production and productivity of rice ought to be augmented. For a crop improvement programme to get off the ground, recognising the genetic variability that already exists in the crop is crucial. The plant breeder can find characteristics for efficient selection with the use of information on the heritability, genetic advance, and association of traits. While selection is made exclusively on yield contributing attributes, heritability and genetic progress are essential selection factors. Heritability estimates combined with genetic advance are often more useful in forecasting gain under selection than heritability estimates alone, it is incredibly important to evaluate the relationship between yield and yield components, as established by correlation analysis. Path co-efficient analysis determines the amount of influence of each yield component characteristic on yield into direct and indirect effects. This study is directed to assess variability, heritability, genetic advance, correlation and path coefficients in some promising genotypes for producing high yielding hybrids or varieties with good amount of nutritional qualities.

MATERIALS AND METHODS

The genotypes used in this study were 14 popular and 19 traditional Tamil Nadu cultivars obtained from TRRI and the Nel Jayaram Institute- Kudavasal. The names of these genotypes are listed in Table 1. The experiment was conducted out at the Plant breeding farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram 608 002, where the genotypes were planted in three replications of 10 rows each, with 15 plants in each row. Each hill keeps a single seedling**.** Agronomic recommendations were followed. Observations on numerous quantitative characteristics were made on a single plant basis in ten randomly selected genotype plants for characters *viz*., days to 50 % flowering, plant height, total number of tillers per plant, number of productive tillers per plant, panicle length, number of grains per panicle, 1000 grain weight and grain yield per plant. TNAUSTAT software was used to perform correlation co-efficient and path co-efficient analysis and different genetic parameters such as genotypic and phenotypic variance, GCV and PCV, heritability, genetic advance and genetic advance as percentage of mean were estimated by using the following formula given by respective authors. The genetic and phenotypic variations were computed using the approach described in (Johnson et al. 1955). The genotypic and phenotypic variation co-efficient were estimated using (Burton, 1952). The approach given by was used to calculate heritability in the broad sense (h^2b) (Johnson *et al*., 1955). The genetic progress was calculated using the formula provided by (Johnson *et al*., 1955). The Al-Jobouri *et al*. (1958) methodology was used to determine the phenotypic and genotypic correlation coefficients among all factors under consideration, and the Dewey and Lu method was used to complete the route analysis (1959).

RESULTS AND DISCUSSION

Genetic variability research reveals information about the amount of variability in a population. Phenotypic variance is the magnitude of variation caused by differences in phenotypic values, whereas genotypic variance is the amount of variation caused by variations in genotypic values. To compare variability among distinct traits, the coefficients of variation expressed at both the genotypic and phenotypic levels were used. The heritability estimate aids in identifying the proportion of variation that is heritable. As a result, the genotypic coefficient of variation (GCV) and the phenotypic coefficient of variation (PCV) can help to determine the degree of variability in genotypes. While heritability with a significant genetic advance as a proportion of the mean would be optimal (Singh *et al*., 2011). The genotypic and phenotypic co-efficients of variation should be graded as high, moderate, and low, according to Siva

Subramanian and Menon (1973). Figure 1 depicts estimated PCV, GCV, heritability, and genetic progress as a percentage of mean for several rice attributes. ANOVA indicated extremely significant differences in all of the characteristics tested across the 33 germplasm lines (Table 2).High PCV and GCV were found for traits such as grain yield per plant and plant height by researchers Islam *et al*., (2015), Aditya and Anuradha (2013). While Patil (2014), Singh *et al.* (2014), Prasad *et al*. (2017), and Battachraya and Chakraborty (2019) all found high GCV and PCV for grain yield per plant and number of productive tillers per plant. Amegan *et al*., (2020) obtained a greater GCV for panicle length. For plant height, high estimations of GCV were observed, and these findings were synchronised with Vanisree *et al* (2013). The characteristics total number of tillers per plant and panicle length have moderate PCV and GCV. In order to increase efficiency, the estimated genetic advance, in addition to heritability, must be regarded as a tool for selection in the breeding programme. Genetic advance represented as a percentage of the mean is a measure of genetic gain under selection. For characters such as 1000 grain weight, plant height, days to 50% flowering, grain yield per plant, total number of tillers per plant, and panicle length, the following authors found similar results: Sanghera *et al*., (2013) for days to 50% flowering, Subuddhi and Dikshit (2009), Pandey *et al*., (2009) and Sanghera *et al*., (2013) for plant height. The heritability and genetic advance of 1000 grain weight, plant height and grain yield per plant was high for high genetic advance results reported were similar to study conducted by Prasad *et al*., (2017) (Table 3). The current study's findings on variability, heritability, and genetic advance revealed potential for improving rice grain yield through selection, using parameters such as the genetic coefficient of variation, heritability, and genetic advance as % of mean. These parameters are critical when developing an efficient rice breeding programme, because when there is enough genetic variation, breeders can take advantage of additive gene effects, transgressive segregation, and heterosis to improve yield (Rashid *et al.,* 2017).

The degree of association and relationship between two variables is measured by the correlation coefficient. It is useful in plant breeding since it allows for indirect selection. The examination of correlations between multiple characters may aid plant breeders in understanding by what means improving one particular character that leads to changes in other characters**.** At both the phenotypic and genotypic levels, there was no significant association between days to 50% flowering and any other trait. Plant height registered significant positive association with number of productive tillers per plant, 1000 grain weight and grain yield per plant. Similar results were reported by Chandra *et al.,* (2009).Total number of tillers per plant had positive significant association with panicle length. Number of productive tillers per plant had registered significant positive association with number of grains per panicle and 1000 grain weight at genotypic level and grain yield per plant at phenotypic level. These results were in agreement with (Chandra *et al.,*2009) for grain yield per plant and Nanda *et al.,* (2019), Priyanka *et al.,* (2019) and Thippani *et al.,* (2017) findings were similar to study done for number of grains per panicle. Number of grains per panicle had positive significant association with grain yield per plant. The findings of current experiment were in agreement with the findings of Chandra *et al.,* (2009).At both the phenotypic and genotypic levels, number of grains per panicle demonstrated a positive relationship with grain yield per plant results were as same as Saha *et al.,* (2019) findings. Character such as 1000 grain weight demonstrated a positive correlation with solely grain yield per plant (Table 4). The current study was same as findings done by following authors Adhikari *et al.,* (2018),Kumar *et al.,* (2018b), Bhujel *et al*., (2018).Path analysis provides a way for categorising the correlation co-efficient into direct and indirect effects and determining the relative relevance of the contributing components involved Grain yield per plant was directly affected by character such as 1000 grain weight and number of productive tillers per plant. Grain yield per plant was less positively influenced by the characters *viz*., number of grains per panicle and total number of tillers per plant. The findings were similar to the results given by Saha *et al.,* (2019); Vennila and Palaniraja (2019) and Sarker *et al.,* (2014) had direct positive effects on total number of tillers per plant over yield**,** other factors such as days to 50% flowering, plant height, and panicle length all had a direct detrimental impact on grain yield per plant (Table 5). Likewise, Nanda *et al.,* (2019) and Minnie *et al.,* (2013) showed positive significant indirect effect on grain yield per plant *via*, number of productive tillers per plant, panicle length and total number of grains per panicle.

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Table 1. List of genotypes selected for variability studies

S.No	Genotype code	Name of the genotype	Source of the material
1	G1	CO 5	Tamil Nadu Rice Research Institute, Aduthurai
2	G2	White Ponni	Tamil Nadu Rice Research Institute, Aduthurai
3	G3	ADT 38	Tamil Nadu Rice Research Institute, Aduthurai
4	G4	TRY 2	Tamil Nadu Rice Research Institute, Aduthurai
5	G5	ABT 39	Tamil Nadu Rice Research Institute, Aduthurai
6	G6	CO 52	Tamil Nadu Rice Research Institute, Aduthurai
7	G7	CO 43	Tamil Nadu Rice Research Institute, Aduthurai
8	G8	ADT 46	Tamil Nadu Rice Research Institute, Aduthurai
9	G9	ADT 54	Tamil Nadu Rice Research Institute, Aduthurai
10	G10	TRY 1	Tamil Nadu Rice Research Institute, Aduthurai

11	G11	TRY 3	Tamil Nadu Rice Research Institute, Aduthurai
12	G12	BPT 5204	Tamil Nadu Rice Research Institute, Aduthurai
13	G13	ASD19	Tamil Nadu Rice Research Institute, Aduthurai
14	G14	CR 1009	Tamil Nadu Rice Research Institute, Aduthurai
15	G15	Karupu Kavuni	Nel Jayaram – Paddy Research centre, Kudavasal
16	G16	Salem Sanna	Nel Jayaram – Paddy Research centre, Kudavasal
17	G17	Sivan Samba	Nel Jayaram – Paddy Research centre, Kudavasal
18	G18	Poongar	Nel Jayaram – Paddy Research centre, Kudavasal
19	G19	Soorna Masuri	Nel Jayaram – Paddy Research centre, Kudavasal
20	G20	Seeraga Samba	Nel Jayaram – Paddy Research centre, Kudavasal
21	G21	Kullakar	Nel Jayaram – Paddy Research centre, Kudavasal
22	G22	Aathurkichidi Samba	Nel Jayaram – Paddy Research centre, Kudavasal
23	G23	Sigappukar	Nel Jayaram – Paddy Research centre, Kudavasal
24	G24	Sivapu Kavuni	Nel Jayaram – Paddy Research centre, Kudavasal
25	G25	Kantha Saala	Nel Jayaram – Paddy Research centre, Kudavasal
26	G26	Mottakur	Nel Jayaram – Paddy Research centre, Kudavasal
27	G27	Kalsar	Nel Jayaram – Paddy Research centre, Kudavasal
28	G28	Madumulungi	Nel Jayaram – Paddy Research centre, Kudavasal
29	G29	Sempuli Samba	Nel Jayaram – Paddy Research centre, Kudavasal
30	G30	Kalurundai	Nel Jayaram – Paddy Research centre, Kudavasal
31	G31	Illupai PooSamba	Nel Jayaram – Paddy Research centre, Kudavasal
32	G32	Mapillai Samba	Nel Jayaram – Paddy Research centre, Kudavasal
33	G33	Kaatuyanam	Nel Jayaram – Paddy Research centre, Kudavasal

Table 2. Analysis of variance for eight quantitative characters

source	df	MSS							
		Days to 50% flowering (days)	Plant height(cm)	Total no of tillers per plant	No of productive tillers per plant	Panicle length(cm)	No of grains per panicle	1000 grain weight(g)	Grain yield per plant (g)
Replication	2	348.16	30.13	16.53	0.86	8.41	13.66	0.22	25.72
Genotype	32	1708.39**	3565.20**	35.61**	13.58**	19.61**	1018.26**	67.48**	749.21**
Error	64	27.99	33.29	1.75	1.46	1.39	203.98	0.13	33.58

**significant at 1% level

Table 3. Variability studies for eight quantitative characters

SI. No	Characters	V^{Ph}	V_g	PCV (%)	GCV (%)	h^2	Genetic advance as % of mean
1	**Days to 50 % flowering**	588.12	560.13	21.77	21.25	95.24	42.72
2	**Plant height**	1210.59	1177.30	34.00	33.53	97.24	68.12
3	**Total number of tillers per plant**	13.04	11.28	15.31	14.24	86.55	27.29
4	**Number of productive tillers per plant**	5.50	4.04	20.96	17.96	73.42	31.71

5	**Panicle length**	7.45	6.08	11.72	10.58	81.52	19.69
6	**Number of grains per panicle**	475.41	271.42	12.71	9.61	57.09	14.95
7	**1000grain weight**	22.58	22.44	22.59	22.52	99.38	46.26
8	**Grain yield**	272.13	238.54	39.83	37.29	87.65	71.93

Abbreviation: PCV- phenotypic coefficient of variation, **GCV**- Genotypic coefficient of variation, **h^2**- heritability

Table 4. Phenotypic and Genotypic correlations for quantitative characters

Characters		DFF	PH	TNT	NPT	PL	NGPP	TGW	GYPP
DFF	P	1.000	0.1686	-0.0082	0.1073	0.0170	-0.0889	-0.0673	-0.0204
	G	1.000	0.1759	-0.0268	0.1432	0.0351	-0.0895	-0.0673	-0.0087
PH	P		1.000	0.2058	0.5357**	0.1900	0.1861	0.4114*	0.5372**
	G		1.000	0.2190	0.6590**	0.2103	0.2633	0.4170*	0.5956**
TNT	P			1.000	0.2088	0.3746*	0.1358	0.1587	0.2293
	G			1.000	0.2963	0.4093*	0.1811	0.1755	0.2813
NPT	P				1.000	0.2258	0.2538	0.3271	0.7493**
	G				1.000	0.3229	0.3585*	0.3775*	0.7526**
PL	P					1.000	0.0814	0.1558	0.2084
	G					1.000	0.0445	0.1757	0.2547
NGPP	P						1.000	0.1957	0.4960**
	G						1.000	0.2673	0.5060**
TGW	P							1.000	0.8158**
	G							1.000	0.8703**

**significant at 1% level*Significant at 5% level

Abbreviation-DFF-Days to 50% flowering; **PH-**plant height; **TNT**-Total number of tillers per plant; **NPT-** Number of productive tillers per plant; **PL**- panicle length; **NGPP**-Number of grains per panicle; **TGW**-1000 grain weight; **GYPP**- Grain yield per plant.

Table 5. Path coefficient analysis for quantitative characters

Characters	DFF	PH	TNT	NPT	PL	NGPP	TGW	GYP
DFF	**-0.0099**	-0.0049	-0.0003	0.0665	-0.0006	-0.015	-0.0446	-0.0087
PH	-0.0017	**-0.0277**	0.0021	0.3063	-0.0036	0.0442	0.2761	0.5956
TNT	0.0003	-0.0061	**0.0097**	0.1377	-0.0069	0.0304	0.1162	0.2813
NPT	-0.0014	-0.0183	0.0029	**0.4648**	-0.0055	0.0602	0.2499	0.7526
PL	-0.0003	-0.0058	0.004	0.1501	**-0.0169**	0.0075	0.1163	0.2547
NGPP	0.0009	-0.0073	0.0018	0.1666	-0.0008	**0.1678**	0.177	0.506
TGW	0.0007	-0.0116	0.0017	0.1754	-0.003	0.0449	**0.6621**	**0.8703**

Abbreviation- DFF-Days to 50 % flowering; **PH-**plant height; **TNT**-Total number of tillers per plant; **NPT-** Number of productive tillers per plant; **PL**- panicle length; **NGPP**-Number of grains per panicle; **TGW**-1000 grain weight.

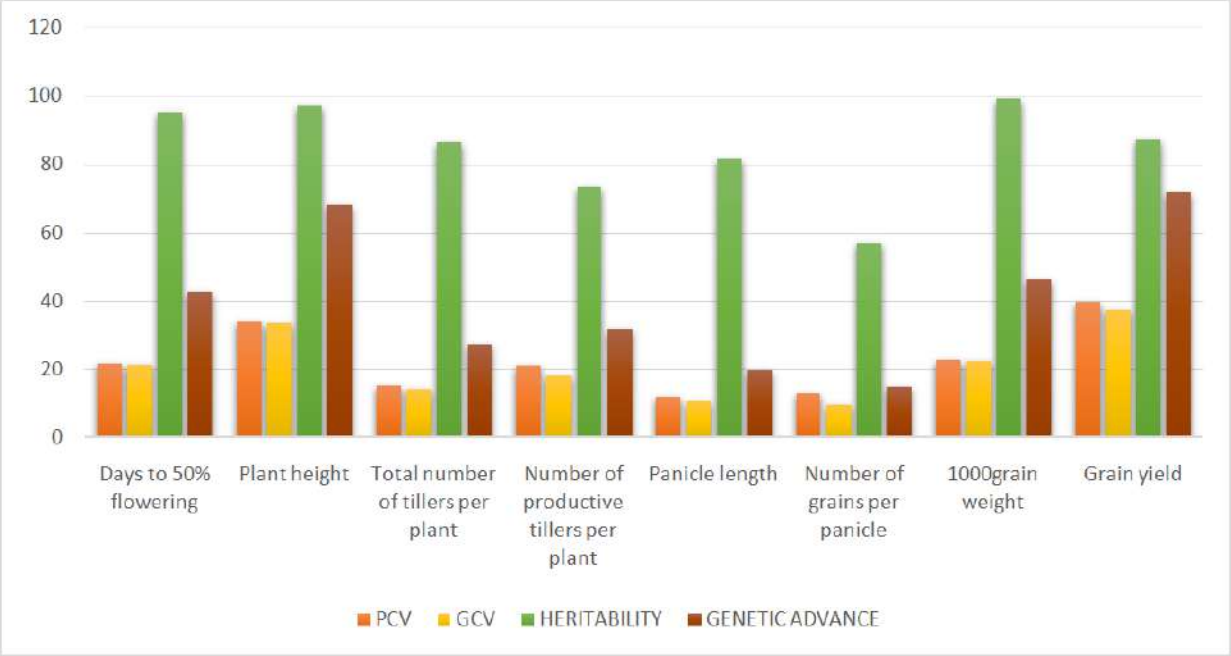

Fig. 1. Magnitude of variability of 33 rice genotypes for quantitative characters

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RESEARCH ARTICLE

Study of Knowledge, Attitude and Awareness of using Mobile Phones among Rural Tertiary Health Care Workers during Covid-19 Pandemic

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ABSTRACT

Coronavirus diseaseis an acute respiratory disease caused by a novel coronavirus and was first detected in Wuhan, China. The key transmission routes are oral and nasal droplets or exposure to contaminated surfaces. Healthcare professionals itself may act as a carrier between infectious patients in hospitals and the general population in transmitting the disease. One of the modes of the transmission could be use of mobile phones. Hence the following study was aimed to know the knowledge, attitude and awareness of using mobile phones among rural tertiary health care workers during covid-19 pandemic. After obtaining institutional ethical clearancethis cross sectional study was conducted using questioners. 264 healthcare workers participated in this study. Out of which post graduate students were 130 (49.2%), consultant were 32 (12.1%) and nurses were 102 (38.7%). 128(48.64%) people felt covid-19 can spread by using mobile phones, 236 (89.68%) people felt sanitization of mobile phones is must. All the participants were using mobile phones pre pandemic and only 5(1.9%) people were using more than one but increased to 16 (6%) during pandemic. Around 250(95%) people use same mobile phone for both hospital and in outside hospital and 180 (68.4%) people agreed that they sanitize the mobile after coming to home. Applying hand sanitizer was practiced by 104 (39.52%) healthcare workers and was commonest form. Mobile phones are convenient and most commonly used method of communication, but it may act as potential source of spreading Covid-19. Separate communication method or safe technique of disinfection like ultraviolet light exposure must be considered for mobile.

Keywords: Covid-19, doctor, mobile phones, nurse, Sanitizer

INTRODUCTION

Coronavirus disease (COVID-19) is an acute respiratory disease caused by a novel coronavirus and was first detected in December 2019 in Wuhan, China. As covid did spread to more than 200 countries and it was declared as a global pandemic. The main transmission routes are oral and nasal droplets or exposure to contaminated surfaces. The virus could persist on inanimate surfaces such as metals, glass or plastic for up to 9 days and may survive for 14 days on certain surfaces. Fortunately inactivated or destroyed by surface disinfection procedures. The washing hands and regular disinfection practices should reduce the possibilities of transmission of the coronavirus by this potential route of infection. For any infectious disease which spreads by contagious route, one has to identify the possible technique which will interrupt, prevent and control further disease transmission. Healthcare professionals may act as a bridge between infectious patients in hospitals and the general population in transmitting the disease if proper care of decontamination is not taken. The healthcare worker is depends upon many materials which is common in use of both within the hospital premises and in public which may facilitate such disease transmission. These include clothes, water bottle, electronic gadget mainly mobile phones. It is important for healthcare professionals to use mobile phones in the hospital and other health and care settings for the necessity of communication. The health professionals extensively use mobile phones for various reasons like to communicate to other health professional or to speak to the relatives, to update themselves with latest guidelines, treatment protocols, to maintain log book, to take pictures for medico legal evidence etc. The use of mobile phones is very common among health workers which range from once in every 15 min to once in 2 hours. However, one should not forget that mobile phone surfaces are a high-risk surface, which can directly come in contact with the face or mouth, while talking over phone. The mobile phones may itself can act as reservoir for microorganisms and may face multi drug resistance organisms.[1,2] Although the healthcare workers are well educated for proper hand wash and its importance to limit the spread of the covid, there is a high chance that mobile phone may act as a source of carrying covid virus. Hence the following study was aimed to know the knowledge, attitude and awareness of using mobile phones among rural tertiary health care workers.

MATERIALS AND METHODS

This cross sectional study was conducted after obtaining institutional ethical clearance. The questions based on knowledge, attitude and awareness of using mobile phones during covid pandemic was prepared as shown in table 1 to 3. These questions were distributed to the healthcare workers of our rural tertiary care workers who were willing to give written informed consent to be the part of the study. A total of 264 healthcare workers participated in this study. The results are compiled in Microsoft excel and analysed accordingly.

RESULTS

Out of 264 healthcare workers, 113(42.94%) were females and 151 (57.38%) were males. In the present study 33 (12.54%) consultants, 110(41.8%) post graduate students, 101 (38.38%) nurses and 30 (11.4%) patient attendants participated. All participants were aware about covid pandemic, Around 128 (48.64%) people were sure that covid spread can occur through mobile use, while 59 (22.42%) people felt it may act as one of the mode of carrier to spread infection. 236 (9.88%) healthcare workers accepted that the mobile sanitization is must while 18 (6.84%) people thought it may be considered. 10 (3.8%) health care workers felt mobile sanitization is not at all required. During pre-pandemic period all participants were using mobile phones in hospital, in that 242 (91.96%) people were using only one mobile phone, and 22 (8.36%) were using more than one mobile phone. During covid pandemic252(95.76%) healthcare workers were still using mobile phones in hospital. Out of which 250(95%) people kept same mobile phone in both hospital and in their house. It was observed that only 196 (74.48%) healthcare workers were sanitizing the mobile phone after reaching home and most common method used to sanitize mobile phone was by application of hand sanitizer (118, 44.84%). Among all healthcare workers, 132 (50.16%) people felt that, telecommunication with

landline is a safer option than mobile, while 46(17.48%) people felt it "may be" safe, rest others were in agreement that mobile phone is safe. Only 80 (30.4%) healthcare workers felt complete stoppage of use of mobile phone will halt the transmission of mobile phones.

DISCUSSION

In the modern times the mobile phones are playing a vital role in day to day life. This is due to various features provided by the mobile producing companies. To enlist some of the important features are, tele consultation in which the consultant can exactly see the patient by video call, getting various lab reports as soon as the laboratory tests are ready, electrocardiogram (ECG) and point of care reports. The mobile phones also help to call other healthcare worker in case of emergency. All these are helping to treat the patient with no delay. The mobile phones are also helping to search a particular guidelines or treatment related to the diseases. Certain mobile phones are also having the features like recording ECG or peripheral oxygen saturation and measurement of pulse rate. Like stethoscope the mobile phones unfortunately carry the risk of transmission of infectious organism between the patients or from the patient to healthcare workers[3,4,5].The mobile phone has added risk of transmission of from patient to family members if proper care is not taken. In our study all participants were aware of the covid pandemic. It is expected that almost all participants will continue to use mobile phones due to the availability of various features as mentioned above. Although the use of mobile phones in covid area is not a problem, but the risk of transmission was more worry some. Only 128(48.64%) healthcare workers felt that the transmission could occur due to use of mobile phones. Various advantages are associated with mobile phone but have a major disadvantage like carrying infection and cross transmission from patient to patient or patient to healthcare workers and healthcare workers to family members. One of the major disadvantage is it may distract the concentration from patient care. Hence mobile phones should be banned atleast in the critical care area after ensuring proper telecommunication facility is made available. In other part of the hospital one can make restriction for the usage of mobile phones[6,7].In our study only 80 healthcare workers felt complete stoppage of mobile phone use in hospital area can help in curtailing healthcare worker related covid spread. Most of the participants had opinion about sanitizing mobile is must after the duty hours. It is not very well discussed by the mobile companies how to sanitize the mobile safely.

There are many infections other than corona virus which can spread by mobile phones due to contamination. Although various materials like antibiotic coated mobile covers are available but may not act full proof against all organisms, especially viruses [8,9].With the covid pandemic the awareness of decontamination and sanitizing mobile has come among healthcare workers. Although it is not a good technique to sanitize the mobile with hand sanitizers, as it is liquid which may enter into the device and spoil it. It also has added disadvantages like, due to presence of moisturizer it becomes sticky and capture the dust and in turn it may block the small holes of speakers or receiver ends. With best of experience authors recommend following method to maintain hygiene of mobile phone. Disconnect any charging point and switch off the mobile before cleaning. Use soft cloth and slightly dampen with a small quantity of biocide and gently wipe front and back side. Cautions to be taken are, not to squeeze any liquid into any holes of mobile and spray the content directly on the mobile. One can consider using of portable UV chamber after decontamination of mobile phones as a method of sanitization. The cost effectiveness is a major challenge for UV chambers[10]. Twenty two healthcare workers who felt use of mobile phones in hospital may possibly carry the infection and decided to have additional mobile phone in the nonhospital area. The problem which could arise multiple mobile is multiple numbers and added expense to maintain sim card of service provider. To reduce the cost of mobile and sim card one can think of forwarding the call to hospital land line. But such call forwording may have certain disadvantage like, difficult to make it portable, bulky, unable to access internet and privacy may get compromised.

LIMITATION OF THE STUDY

In this study we did not ask for how many mobile phones got damaged or became nonfunctional with the use of sterilization methods mentioned. We did not study the amount of use of sanitizer and the contact period for sanitizing.

CONCLUSION

Mobile phones are the most convenient and commonly used for the communication, however one should not forget that it can act as a potential source for spreading Covid-19 infection. Separate communication method like use of landline phone with loudspeaker facility can be considered. The government agencies and the World Health Organization must consider developing awareness of possible spread of covid virus and must come up with guidelines for it.

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Table 1: The questions related to socio demographic data

Socio-demographic characteristics			
Age			Sex
Profession	Doctors	Post Graduate	
		Faculty	
	Nursing Staff		
	Others		

Table 2: The questions related to Knowledge and attitude

Knowledge assessment			
Question	**Yes**	**No**	**Not Sure**
Presently are we facing Covid-19 pandemic?			
Do you feel Covid-19 can spread through usage of mobile phones?			
Do you feel that sanitization can reduce spread of Covid-19 ?			
Attitude towards mobile phone usage			
Question	**Yes**	**No**	**Not Sure**
Before Covid Pandemic were you using Mobile phones?			
How many mobile phones were you using before Covid-19 pandemic?	• One • More than one		
During Covid-19 pandemic are you using mobile phone ?			
How many mobile phones were you using in covid times?			
During covid – 19 pandemic have you added additional phone from the existing?			
Do you use the same mobile phone in house and in hospital ?			
Do you sanitize the mobile phone before coming home?			
If yes , then how do you sanitize the mobile phone?	1. Just cleaning with plain water 2. Clean with soap and water 3. Alcohol swabs 4. Application of hand sanitizer to mobile phone 5. I put transparent mobile cover and throw it everyday 6. I use UV chamber to sterilize the mobile phone 7. I use separate mobile phone for hospital so not required 8. Some other method		

Table 3: The questions related to awareness

Awareness assessment			
Question	**Yes**	**No**	**Maybe/Not applicable**
Are you aware that complete stoppage of using mobile phones can decrease the risk of transmission significantly?			
Do you think that telecommunication through landline is safer than using your own mobile phones in hospital area?			

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RESEARCH ARTICLE

Effect of Conventional, Non-Conventional Organic Sources Industrial by-Products for Incubation Study, and Yield by Maize

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ABSTRACT

The incubation experiments was conducted with organic sources like Vermicompost. Municipal solid waste compost and industrial by products like rice husk ash, lignite fly ash with chemical fertilizers are used to study the release pattern of nutrients. The soil was collected from Varagurpettai village having clay loam, pH 7.6, EC 0.3 dSm^{-1} (*Typic haplusterts*). The treatments include control (135:62.5 50 kg ha^{-1}), Municipal solid waste compost (@ 5 t ha^{-1}, 10 t ha^{-1}), Vermicompost (@ 2.5 t ha^{-1}, 5 t ha^{-1}) Bagasse ash (5 t ha^{-1}, 10 t ha^{-1}), Lignite fly ash (5 t ha^{-1}, 10 t ha^{-1}). Soil samples at 30, 60 and 90 DAI were taken and analyzed for pH, EC, organic carbon, NPK and micronutrients. Pot experiment was conducted in Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University to evaluate yield of maize. The same set of treatments in pot culture were followed as per incubation study. The results showed that highest nutrient release pattern and yield of maize were registered in treatment receiving 75% RDF + Vermicompost @ 5 t ha^{-1}.

Keywords: Maize, Incubation study, Vermicompost and Yield.

INTRODUCTION

Maize (*Zea mays* L.) known as queen of cereals, also called corn is one of the most important cereal crops of the world. In India maize crop stand up as the third cash crop after wheat and rice. Maize is not only used as human food and animal feed, but is as well commonly used in several other industries as a raw material. In India maize cultivation is taken up in an area of 9.47 million hectares with an annual production of 28.72 million tones (Agriculture Statistics at a Glance,

2018). In any country or state, waste generation rates are affected by socio-economic development, degree of industrialization, urbanization, climatic and geographical conditions. It is difficult to define what are the population exposed to the direct or indirect effects of inadequate municipal solid waste management because information and monitoring systems on the health and the environment do not consider the collective aspect of the populations and there are no sufficient and reliable epidemiological data available. Municipal solid waste management system involves various activities like storage, collection, transportation, disposal *etc.* Waste management is a problem in urban and rural areas. Waste minimization is a methodology used to achieve waste reduction, primarily through reduction at source, but also including recycling and rescue of material. Composting is a biological process of decomposition carried out under controlled. Conditions of ventilation, temperature, moisture and organisms is the waste themselves that convert waste into humus – like material by acting on the organic portion of the solid waste. Composting is the controlled biological process to turning organic waste into soil conditioner. In nature, organic matter such as wood, paper, animal waste and plant material is decomposed by bacteria (Shamin Banu ad Kanagasabai, 2012).

Pressmud is a by-product in sugar industry. Pressmud is heavily polluting and also harmful effects on the ambient environment. It generates intense heat (65°C) and foul odour. Its natural decomposition takes long times. These organic wastes are converted to compost by earthworm's action over a certain period of time. By introducing earthworms into heaps of pressmud feed on organic waste and the earthworms get acts as a bioreactor and vermicates are produced. Vermicompost maintains a steay mineral balance, improves nutrients availability for rejuvenating the soil, in addition of reduction of pathogenic organisms too (Geeta Utekar and Hanamantrao Deshmukh, 2016). Lignite fly ash is being generated by the combustion of coal in over 82 thermal power plants. Its generation is estimated to cross over 140 million tones by 2020. The huge quantity of lignite fly ash poses problem for its storage, requiring around 30,000 hectares of land for its safe disposal. The lignite fly ash of NLC serves as supplementary source of essential plant nutrients and is also effective in the reclamation of waste degraded land and mine spoil (Saranraj, 2015). Bagasse ash is a good source of micronutrients like Fe, Mn, Zn and Cu and also high concentration of P and K (Dotaniya *et al.,* 2016). The objectives are to study nutrient release pattern of various organic manures (municipal solid waste compost and vermicompost) and industrial by products (bagasse ash and lignite fly ash) in soil through incubation experiment, to study the direct effect of conventional and non-conventional organic sources, industrial by-products and fertilizers on the maize cob and stover yield.

MATERIALS AND METHODS

The study involved soil incubation experiment and pot experiment at Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University with maize as a test crop. Materials used for incubation and pot experiment municipal solid waste compost, vermicompost, bagasse and fly ash.

Collection of soil sample

The soil samples were collected from Varagurpettai village, Chidambaram taluk of Cuddalore district, Tamil Nadu to conduct, incubation and pot experiment.

Laboratory incubation experiments and treatment details

An incubation experiment was conducted with an objective of studying the effect of nutrient management on the release pattern of nutrients from conventional and non-conventional organic sources, industrial by-products and inorganic fertilizer 200 g of 2 mm sieved soil sample was filled in 250 ml (depth 9 cm, diameter 21 cm) plastic container. The treatment details are given below. Each treatment was replicated thrice. The soil was incubated at room temperature for 90 days at field capacity. The design followed was Completely Randomized Design (CRD).

Treatment details of the incubation experiment

T_1 – Control – 100% RDF (135:62.5:50 kg ha^{-1})

T_2 – 75% RDF + Municipal solid waste compost @ 5 t ha^{-1}

T_3 – 75% RDF + Municipal solid waste compost @ 10 t ha^{-1}
T_4 – 75% RDF + Vermicompost @ 2.5 t ha^{-1}
T_5 – 75% RDF + Vermicompost @ 5 t ha^{-1}
T_6 – 75% RDF + Bagasse ash @ 5 t ha^{-1}
T_7 – 75% RDF + Bagasse ash @ 10 t ha^{-1}
T_8 – 75% RDF + Lignite flyash @ 5 t ha^{-1}
T_7 – 75% RDF + Lignite flyash @ 10 t ha^{-1}

The soil samples were drawn at 30, 60 and 90 DAI and analysed for pH, EC, organic carbon, available NPK content and DTPA extractable Fe, Mn, Zn and Cu content. These treatments were studied in the pot experiment to evaluate their efficiency in yield of maize. The same set of incubation treatments were followed in pot experiment also. The experiment was conducted in a completely randomized block design (CRD) with the following nine treatments. Grain yield (g pot^{-1}) At physiological maturity, cobs from the pot were harvested. The mean grain yield obtained in each treatment was expressed in g pot^{-1}

Stover yield (g pot^{-1})

After drying of straw, the stover yield from each pot was recorded and yield per hectare was calculated. The mean stover yield obtained in each treatment was expressed in g pot^{-1}.

Analysis of soil sample

Soil sample were collected just before the start of the pot experiment and at harvest to determine the various physico-chemical characteristics and nutrient status of the soil. The collected soil samples were air dried in shade, ground with wooden mallet, passed through 2 mm sieve and stored in polythene bags. These samples were analyzed for pH, EC, organic carbon, available NPK, DTPA extractable Fe, Mn, Zn and Cu. Standard procedures followed for the analysis of soil samples are listed in Table 1.

RESULTS AND DISCUSSION

Physico-chemical properties of experimental soil

The composite soil at 0-15 cm collected from Varagurpettai were analyzed for various physico-chemical properties (Table 3). The textural composition of soil was clay loam. The experimental soil of Varagurpettai comes under the taxonomical classification of *Typic haplusterts*. The cation exchange capacity was 22.1 [cmol(P^+) kg ha^{-1}]. The soil pH was 7.6 with EC of 0.31 dSm^{-1}. The organic carbon content was 4.5 g kg^{-1}. The available nitrogen, phosphorus and potassium content of the soil were 235.2, 38 and 226.4 kg ha^{-1} respectively recording low, high and medium status in soil fertility. The exchangeable Ca and Mg were 10 and 4.4 [cmol(P^+) kg ha^{-1}) and available sulphur was 11.4 mg kg^{-1} and status was medium in soil. The DTPA extractable Fe (13.2 mg kg^{-1}), Mn (14.1 mg kg^{-1}) and Cu (2.8 mg kg^{-1}) and status were high for Fe, Mn and Cu. The DTPA extractable Zn (0.74 mg kg^{-1}) status was low.

Incubation study

Soil reaction

The application of conventional, non-conventional organic sources recorded lowest pH value at all three stages of incubation period (Table 4). Among organic sources, application of 75% RDF + Vermicompost @ 5 t ha^{-1} (T_5) recorded the lowest pH of the soil at all three stages of incubation period. The lowest pH values observed with this treatment were (T_5) 7.1 at 30 DAI, 7.0 at 60 DAI and 6.9 at 90 DAI. Among the industrial by-products the highest pH at all stages with 75% RDF + Bagasse ash @ 10 t ha^{-1} (T_7) registered 8.1 at 30 DAI, 8.2 at 60 DAI and 8.3 at 90 DAI. It is obvious that improvement in chemical properties of soil is the prerequisite for better crop nutrition. All the conventional, non-conventional organic sources and industrial by-products used in the incubation experiment significantly improved the chemical properties of soil. In the incubation experiment carried out, the conventional organic sources greatly reduced the soil reaction (pH) of alluvial soil. The initial soil had pH of 7.6. A reduction in soil pH value of 6.9 at 90 DAI was noticed due to application of 75% + Vermicompost @ 5 t ha^{-1} (T_5). The favourable reduction in pH could be attributed to the prolonged decomposition of added conventional organic sources. Decline in soil pH can have positive impact on availability of nutrients such as phosphorus, zinc, iron and manganese. The addition of vermicompost to clay soil increased all physico-

chemical parameters except for pH. Similar results were observed in Ramasamy *et al.* (2011). Among industrial by-product, the increase in pH value of 8.3 was recorded in the treatment T_5 (100% RDF + Bagasse ash @ 10 t ha^{-1}) it is due to amendment of bagasse ash had some effect on soil bulk density. The results are in conformity with the findings of Sabanoor *et al.* (2016).

Electrical conductivity (EC)

The non-conventional organic source (Table 4) increased in soil EC (0.67 dSm^{-1}) of at 90 DAI application of 75% RDF + Municipal solid waste compost @ 10 t ha^{-1} (T_3) which normally increase the soil EC due to its salt content in municipal solid waste compost. Conductivity is a measure of the current carrying capacity thus gives the idea of soluble salts in the soil. The presence of large amount of ionic substance and soluble salts have resulted in increased value of EC in the municipal solid waste compost treated soil sample in comparison to the control soil sample (Utpal Goswami and Sharma, 2008). Among industrial by-products, the increase in soil EC (0.4 dSm^{-1}) of at 90 DAI is due to application of 75% RDF + Bagasse ash @ 10 t ha^{-1} (T_7). The addition of bagasse ash gradually increased EC value of soil. Increasing EC value of soil is due to addition of bagasse ash may be attributed to the fact the salts from bagasse ash might have dissolved in soil moisture and there by increased the ionic concentration of the soil solution, similar results were noticed in Gaurav Bhusan (2016).

Organic carbon

The organic carbon (Table 4) of the experiment soil was 4.5 g kg^{-1} and rated low. The organic carbon content of the incubated soils was generally influenced by the addition of conventional and non-conventional organic sources. The increase in organic carbon content of soil was in the range of 4.3 to 5.9 g kg^{-1} in the incubation experiment with organic sources. Among treatments 75% RDF + Vermicompost @ 5 t ha^{-1} (T_5) excelled all the treatment by recording 5.2, 5.6 and 5.9 g kg^{-1} at 30, 60 and 90 DAI respectively. Vermicompost is an already decomposed material and will incorporate more organic matter (OM) to the soil, with consequent increase in SOC stock because of the greater OM stability in the compost. Vermicompost is not naked carbon but humus in making containing labile organic mater with stable organic compounds. The increase in organic carbon is due to organic mater and carbon content with the application of compost or vermicompost either combined or not with mineral fertilizers (Oroka Frank Oke, 2015).

$KMnO_4$–N

The data on $KMnO_4$–N (Table 5) varied significantly on 60 and 90 DAI. The available nitrogen of the experimental soil was 117.6 mg kg^{-1} which is rated low. The available nitrogen content of the incubation was greatly influenced by the addition of conventional and non-conventional organic sources as evidenced in to incubation experiment. The increase in available nitrogen content of the soil was in the range of 117.1 to 142.7 mg kg^{-1} in the incubation experiment due to various sources and industrial by-products. The application of 75% RDF + Vermicompost @ 5 t ha^{-1} (T_5) in the incubation experiment excelled all other treatment by recording the available nitrogen content of 142.7 mg kg^{-1} at 90 DAI. The increased N availability with applied vermicompost might be due to the increased decomposition of applied organic manures under favourable soil environment and due to reduced volatilization, leading and denitrifying losses. This was online with findings of Thuy Thu Doan *et al.* (2015). The application of 75% NPK + Municipal Solid Waste Compost @ 10 t ha^{-1} (T_3) recorded the value of 131.7 mg kg^{-1} at 90 DAI. The increase in available N content can be explained by its high concentration of available N at the time of application in MSWC compost. Similar findings were reported by Weber *et al.* (2007).

Olsen-P

The increased Olsen-P content (Table 5) due to application of conventional, non-conventional organic sources and industrial by-products were well evidenced in the incubation experiment. The experiment soil exhibited high status of available phosphorus. The increased availability of phosphorus due to application of conventional and non-congenital organic sources and industrial by-products were in the range of 18.5 to 20.1 mg kg^{-1} at 90 DAI. In the incubation studies municipal solid waste compost, vermicompost, bagasse ash, lignite fly ash were used as sources for supplementing P nutrition. The release of phosphorus from vermicompost was steady through out the duration of incubation experiment. Similar results were observed by Sukhmal Chand *et al.* (2011). In incubation experiment the

application of 75% RDF + Municipal solid waste compost @ 10 t ha^{-1} (T_3) registered to value of 19.4 mg kg^{-1} at 90 DAI. The addition of non-conventional organic source reduces the P-adsorption capacity of soil. Organic acids produced by the mineralization of organic manures might compete for adsorption with P, there by reducing the adsorption sites for P. Secondly dissolved organic matter produced via the mineralization of organic fertilizer might cause soil to replace phosphate more. The enveloping effect of organic fertilizers might reduce P absorption of the soil. This is in result with the accordance of findings of Warman *et al.* (2009).

NH_4OAc-K

Application of conventional, non-conventional organic sources, industrial by-products showed an improvement in the K content of soil (Table 5). In incubation experiment, the highest K availability was observed with the application of 75% RDF + Municipal solid waste compost @ 10 t ha^{-1} (T_3) which registered 114.1 mg kg^{-1} at 90 DAI. The application of Municipal solid waste compost found that potassium content of soil increased with increased rate of application. The municipal solid waste compost produced due to domestic and industrial activity is highly heterogeneous with respect to its chemical properties. It has been reported that municipal solid waste compost can result in increased soil fertility similar results were observed by Monica Ozores-Hampton *et al.* (2011).The application of 75% RDF + Lignite fly ash @ 10 t ha^{-1} (T_9) registered 113.4 mg kg^{-1} of potassium at 90 DAI. The increase in available potassium content with lignite fly ash application might be due to release of potassium present in lignite fly ash. Similar results were also made by Lalram *et al.* (2007).

Micronutrients (Fe, Mn, Zn and Cu)

The application of industrial by-products, conventional and non-congenital compost had a marked influence on available Fe, Mn, Zn and Cu content of the soil (Table 6). Among the different parameters 75% RDF + Flyash @ 10 t ha^{-1} (T_9) recorded higher Fe and Mn content of 20.7 and 18.3 mg kg^{-1}. The amount of extractable of Fe and Mn were significantly increased by fly ash application. The addition of Fly ash @ 10 t ha^{-1} produced large increases in the amount of extractable Fe and Mn in alluvial soil. Similar results were observed by Manisha Basu *et al.* (2009).

Grain yield

The application of 100% RDF + Vermicompost @ 5 t ha^{-1} (T_5) registered highest grain yield 416.8 g pot^{-1}. The highest yield in maize plants exposed to particular concentration of vermicompost may be due to the influence of combined effect of various ingredients of vermicompost such as macro (N, P, K) and micro (Mn, Fe, Zn and Cu) nutrients, plant growth hormones (indole acetic acid, indole butyric acid, naphthalene acetic acid and gibberellic acid), vitamins (vitamin A, B_1, B_2, B_3, C and E). The analysis of physico-chemical parameters showed that though nutritional availability is rich in vermicompost, the plant utilizable quantity differed from one concentration of vermicompost to the other. The higher availability of nutrients especially nitrogen and phosphate in vermicompost and improved soil physical, chemical and biological properties might have contributed to higher yield. The possible for increasing grain yield might be due to increase in ear length, 100 grain weight with the same treatment (T_5) including the effect of humic acid on soil physico-chemical properties of soil and providing a medium for absorption of plant nutrient and improved conditions for soil microorganisms. Similar results were observed by Karki *et al.* (2005). Among the industrial by-products the application of 75% RDF + Flyash @ 10 t ha^{-1} (T_9) registered 270 g pot^{-1}. This is due to the supply of nutrients, conducive physical environment leading to better aeration, increase in soil moisture holding capacity, root activity and nutrient absorption and the consequent complementary effect in flyash have resulted in higher grain yield (Kumari Manimuthuveeral, 2018).

Stover yield

The highest stover yield of 545.9 g pot^{-1} were recorded in application of vermicompost @ 5 t ha^{-1} (T_5). The significant increase in stover yield under these fertility levels appears to be on account of their influence on yield attributes and indirectly in a increase in plant growth. This may be due to the effect of both vermicompost and municipal solid waste compost application (Ashish Shivran *et al.* (2015). The potassium plays a major role in growth as it is involved in assimilation transport and storage tissue development. Similar results were observed by Bhanu Prakash *et al.* (2007).Among the industrial by-products the highest stover yield for flyash (348.3 g pot-1) was recorded in the

treatment (T9) receiving 75% RDF + Flyash @ 10 t ha-1 could be beneficial in improving the soil quality and there by leaching to better availability to nutrients (Chandrakar et al., 2015).

CONCLUSION

In the incubation experiment, T_5 registered lowest pH of 6.9 and highest electrical conductivity of 0.32 dSm^{-1}, organic carbon of 5.9 g kg^{-1}, available soil Nitrogen (142.7 mg kg^{-1}), available soil Phosphorus (20.1 mg kg^{-1}), at 90 DAI. The treatment T_3 recorded available Soil Potassium (114.1 mg kg^{-1}) and DTPA extractable Cu (4.4 mg kg^{-1}). The treatment T_7 recorded highest DTPA extractable Fe (20.7 mg kg^{-1}), DTPA extractable Mn (18.3 mg kg^{-1}), at 90 DAI. The tratment T_7 recorded highest DTPA extractable Zn (0.8 mg kg^{-1}) at 90 DAI. Regarding pot experiment highest grain yield pot experiment highest grain yield (416.89 g pot^{-1}) and stover yield (545.9 g pot^{-1}) were recorded in the treatment T_5.

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Table 1. Methods of analysis of soil

S. No.	Parameters	Methodology	References
A. Mechanical Fraction			
1	Textural fraction	International Pipette Method	Piper (1966)
2	Bulk density particle density and pore space	Measuring cylinder method	Sree Ramulu (2003)
3	Soil colour	Munsell soil colour chart	U.S. Dept. of agriculture Hand Book (2000)
B. Physico-chemical properties			
4	Soil reaction pH	Potentiometry (1:2:5 soil suspension)	Jackson (1973)
5	Electrical Conductivity (EC)	Conductometry (1:2.5 soil suspension)	Jackson (1973)
6	CEC	Neutral normal ammonium acetate method	Jackson (1973)

c. Chemical properties			
7	Organic carbon	Chromic acid wet digestion method	Walkley and Black (1934)
8	Available nitrogen ($KMnO_4$-N)	Alkaline permanganate method	Subbiah and asija (1956)
9	Available phosphorus (Olsen-P)	Ascorbic acid blue method (spectrophotometry)	Watanabe and Olsen (1965)
10	Available potassium (NH_4OAC-K)	1N NH_4OAC extract flame photometry	Standford and English (1949)
11	DTPA extractable Fe, Mn, Zn and Cu	DTPA extractable (atomic absorption spectroscopy)	Lindsay and Norvell (1978)

Table 2. Chemical composition of municipal solid waste compost, vermicompost, bagasse ash and lignite flyash

Materials	OC (g kg^{-1})	Total N (%)	Total P (%)	Total K (%)	DTPA Fe (mg kg^{-1})	DTPA Mn (mg kg^{-1})	DTPA Zn (mg kg^{-1})	DTPA Cu (mg kg^{-1})
Municipal solid waste compost	270.0	1.13	2.92	0.53	14.6	1.1	1.1	211.0
Vermicompost	139.6	1.59	3.43	0.27	175	96.5	24.5	5.0
Bagasse ash	71.5	0.014	0.0052	0.024	267	194	65	55
Lignite flyash	22.5	0.008	0.39	0.48	8100	214	31	22

Table 3. Physico-chemical properties of the experimental soil

A.	Mechanical Properties	Content
1	Clay (%)	38.7
2	Silt (%)	15.7
3	Fine sand (%)	32.4
4	Coarse sand (%)	13.2
5	Textural classification	Clay loam
6	Taxonomical classification	*Typic Haplusterts*
B	Physical Properties	
1	Bulk density (Mg m^{-3})	1.22
2	Particle density (Mg m^{-3})	2.65
3	Pore space (%)	54
C	Physico-chemical properties	
1	pH	7.6
2	EC (dSm^{-1})	0.31
3	CEC [cmol(P^+) kg^{-1}]	22.1
D	CHEMICAL PROPERTIES	
1	Organic carbon (g kg^{-1})	0.45 (Low)
2	Available nitrogen (kg ha^{-1})	235.2 (Low)
3	Available phosphorus (kg ha^{-1})	38 (High)
4	Available potassium (kg ha^{-1})	226.4 (Medium)
5	Exchangeable Ca (cmol(P^+) kg^{-1})	10
6	Exchangeable Mg (cmol(P^+) kg^{-1})	4.4
7	Available sulphur (mg kg^{-1})	11.4 (Medium)
8	Fe DTPA extractable (mg kg^{-1})	13.2 (High)

9	Mn DTPA extractable (mg kg^{-1})	14.1 (High)
10	Zn DTPA extractable (mg kg^{-1})	0.74 (Low)
11	Cu DTPA extractable (mg kg^{-1})	2.8 (High)

Table 4. Effect of conventional, non-conventional organic sources and industrial by-products on soil pH, electrical conductivity and organic carbon in incubation experiment

Treatments	Soil reaction (pH)			Electrical conductivity (dSm^{-1})			Organic carbon (g kg^{-1})		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
T_1	7.5	7.4	7.2	0.30	0.31	0.32	4.3	4.2	4.1
T_2	7.4	7.3	7.1	0.61	0.63	0.66	4.7	5.1	5.3
T_3	7.4	7.3	7.2	0.62	0.65	0.67	4.8	5.2	5.5
T_4	7.3	7.2	7.1	0.53	0.56	0.58	5.1	5.5	5.7
T_5	7.1	7.0	6.9	0.57	0.57	0.59	5.2	5.6	5.9
T_6	8.0	8.1	8.2	0.35	0.37	0.39	4.4	4.7	4.8
T_7	8.1	8.2	8.3	0.36	0.38	0.40	4.5	4.8	4.9
T_8	7.8	7.9	8.0	0.32	0.34	0.36	4.1	4.3	4.3
T_9	7.9	8.0	8.1	0.33	0.35	0.37	4.2	4.4	4.6
Mean	7.6	7.6	7.5	0.44	0.46	0.48	4.5	4.8	5.0
S.Ed.	0.36	0.38	0.40	0.03	0.04	0.04	0.27	0.31	0.29
CD (P=0.05)	0.76	0.80	0.84	0.06	0.08	0.10	0.58	0.67	0.62

Table 5. Effect of conventional, non-conventional organic sources and industrial by-products on alkaline $KMnO_4$–N, Olsen-P and NH_4OAC-K in incubation experiment

Treatments	Soil nitrogen (mg kg^{-1})			Soil phosphorus (mg kg^{-1})			Soil potassium (mg kg^{-1})		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
T_1	117.1	117.9	118.1	18.5	18.6	18.6	111.7	111.9	112.0
T_2	123.5	129.1	130.3	19.0	19.1	19.3	113.7	113.8	114.0
T_3	128.1	130.1	131.7	19.0	19.2	19.4	113.8	113.9	114.1
T_4	130.3	140.7	141.3	19.6	19.8	20.0	112.3	112.4	113.7
T_5	138.1	141.1	142.7	19.7	20.0	20.1	112.5	112.6	113.8
T_6	118.1	118.9	113.5	18.6	18.6	18.7	111.9	112.0	112.1
T_7	118.9	119.1	119.1	18.6	18.7	18.7	112.0	112.1	112.2
T_8	117.9	118.1	118.3	18.7	18.8	18.8	112.9	113.1	13.3
T_9	118.1	118.3	118.5	18.8	18.9	18.9	113.1	113.3	113.4
Mean	123.5	125.9	125.9	18.9	19.0	19.1	112.6	112.7	113.1
S.Ed.	7.07	7.21	7.01	108	1.09	1.10	6.44	6.45	6.46
CD (P=0.05)	14.86	15.16	14.73	2.28	2.29	2.32	13.54	13.57	13.58

Table 6. Effect of conventional, non-conventional organic sources and industrial by-products on iron, manganese, zinc and copper experiment

Treatments	Iron (mg kg^{-1})			Manganese (mg kg^{-1})			Zinc (mg kg^{-1})			Copper (mg kg^{-1})		
	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI	30 DAI	60 DAI	90 DAI
T_1	12.5	12.6	12.7	14.4	14.5	14.6	0.69	0.71	0.72	2.6	2.6	2.6
T_2	13.3	13.5	13.6	14.8	14.9	15.0	0.70	0.71	0.73	3.9	4.1	4.3
T_3	13.5	13.7	13.8	15.0	15.1	15.2	0.71	0.72	0.74	4.0	4.2	4.4
T_4	14.2	14.4	14.5	15.9	16.3	16.4	0.74	0.75	0.76	2.7	2.9	3.0
T_5	14.3	14.7	14.8	16.1	16.3	16.5	0.75	0.76	0.78	2.8	3.0	3.2
T_6	19.4	19.5	19.6	16.5	16.7	16.9	0.82	0.83	0.85	3.4	3.6	3.8
T_7	19.6	19.7	19.8	16.7	16.9	17.1	0.84	0.85	0.87	3.5	3.7	3.9
T_8	20.2	20.4	20.5	17.7	17.9	18.1	0.78	0.80	0.82	3.2	3.4	3.6
T_9	20.5	20.6	20.7	17.9	18.1	18.3	0.79	0.81	0.83	3.3	3.5	3.7
Mean	16.3	16.5	16.6	16.1	16.3	16.4	0.75	0.77	0.78	3.2	3.4	3.6
S.Ed.	0.42	0.42	0.42	0.39	0.40	0.40	0.02	0.02	0.02	0.09	0.10	0.10
CD (P=0.05)	0.88	0.88	0.88	0.83	0.85	0.85	0.04	0.04	0.04	0.20	0.22	0.22

Table 7. Effect of conventional, non-conventional organic sources and industrial by-products on grain yield and stover yield

Treatments	Grain yield (g pot^{-1})	Stover yield (g pot^{-1})
T_1	257.3	392.3
T_2	386.9	499.1
T_3	393.8	512.0
T_4	408.1	534.6
T_5	416.8	545.9
T_6	261.0	334.1
T_7	262.8	336.4
T_8	265.5	342.4
T_9	270.0	348.3
Mean	324.7	420.2
S.Ed.	16.11	20.89
CD (P=0.05)	33.86	43.89

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RESEARCH ARTICLE

Impact Assessment of LULC Pattern around Chilika Lake, Odisha using RS and GIS

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ABSTRACT

In detailed land dynamics research, the analysis of land use land cover (LULC) change has always been a great topic of interest. Most of the previous studies tried to use the conventional method of "net change" analysis to describe the spatio-temporal LULC transformation. This study aimed to give the clear signals of landscape transformation over the last few decades, using the landscapes of Chilika Lake, Odisha. The image change detection conducted with GIS has been proven to obtain comprehensive information on the modifications and conversions of land use land covers as a result of spatio-temporal dynamics of natural and human activities. The detection of possible land use land cover changes, monitoring and evaluation in the Chilika Lake area using landsat TM and OLI data is easily described. It means from 1988 the area coverage decreased then it again increased slowly but compared to 1988 in 2021 water area coverage was low. Study shows that the bare land cover trend line also minimal in 1999 then it increased rapidly in between 1999 to 2010 then from 2010 to 2021 it decreased again. Overall, the bare land trend line nature is decreasing. For other categories from 1988 to 2021 the trend of change is downward. The trend line decreases rapidly. This change is directly or indirectly influenced by demographic changes and the overall change is remarkably influenced by the Chilika lake mouth shifting and silting. The present study will able to build up a systematic and scientific future land use policies and to plan an integrated approach to protect the dynamic ecosystems of the region, while creating the provisions for alternative livelihood options.

Keywords: LULC , mouth shifting, net change, silting, spatio-temporal

INTRODUCTION

Land Use Land Cover (LULC) types and their changes are in the mainstream of Global Change Impact studies. Land cover data documents how much of a region is covered by forests, wetlands, impervious surfaces, agriculture, and other land and water types. Water types include wetlands or open water. Land use shows how people use the landscape – whether for development, conservation, or mixed uses. The changes in the physical characteristics of the earth's surface e.g. deforestation, afforestation, distribution of water bodies, soil and types of vegetation as well as anthropogenic changes such as proliferation of manmade structures are captured in the term land use. Land Use / Land Cover (LULC) generally refer to the categorization or classification of human activities and natural elements on the landscape within a specific time frame based on established scientific and methods of analysis of appropriate source materials. Throughout long geological past, it has been seen that Indian subcontinents were experiencing numerous and different scale endogenetic oscillation. The Great Himalayan region, many fault line regions were witnessed several magnitudes of tectonic movement. These tectonic movements are the main pulling factors to make a huge variety in slope changes and this changing nature of slope can moderate the pattern of LULC (Saha et al., 2017).

STUDY AREA

Our study area is Chilika Lagoon and its surrounding areas. It is situated on the east coast of India and is the largest brackish water wetland of Asia and also a Ramsar site. The lagoon is oriented parallel to the coast between the Eastern Ghats and the Bay of Bengal and is connected to the Bay of Bengal through a 25km long narrow outer channel separated from the sea by a narrow spit (Asthana, 1979).The Chilika lagoon spreads for around 830 Sq. km. along the east coast of India in the Odisha state. Chilika catchment is situated on the east coast of India which covers Puri and Khurda districts adjoining Ganjam district of Odisha state. Its catchment also covers 18 blocks of Puri, Khurda, Nayagarh and Ganjam with an area of 3987 sq. km. (Chilika Atlas, 2007). It has been formed due to the silting action of the Mahanadi river, which drains into the northern end of the lake, and the northerly currents in the Bay of Bengal, which have formed a sandbar along the eastern shore leading to the formation of a shallow lagoon

METHODS

Four cloud-free Landsat scenes with 30m spatial resolution were selected and downloaded for the year 1988 (07/01/1988), 1999(07/12/1999), 2010(25/04/2010) and 2021(08/03/2021) for evaluating the LULC types and their changes. The data was downloaded from the USGS Earth explorer site (https://earthexplorer.usgs.gov). The datasets were pre-processed in open source software QGIS using the Semi-Automatic Classification Plug-in for QGIS . First, the raw DN data for individual bands were converted to radiance values and TOA reflectance. Then the TOA reflectance was converted to surface reflectance using the DOS1 correction. An area of interest (AOI) was created for the Chilika Lake and the adjoining areas and both the scenes were subset to the AOI. Following this, NDVI (Normalized Difference Vegetation Index), NDBI (Normalized Difference Built-up Index) and MNDWI (Modified Normalized Difference Water Index) values are calculated using green, NIR (Near Infrared band), red and SWIR (Short Wave Infrared band) bands of satellite images. In Landsat 5 TM, band 2 is green, band 3 red, band 4 NIR and band 5 SWIR. And in Landsat 8 OLI band 3 is green, band4 red, band 5 NIR and band 6 SWIR. For our change detection we used QGIS 2.16 version and ARCMAP 10.4 version software. Where we performed the NDVI, NDBI and MNDWI indexes and therefore we reclassify the indexes using their threshold values to get the vegetation, bare land and water body layers of those four years. By this a pixel-based comparison was used to identify change information for each of the classes and thus interpret changes using "-from, -to" information from 1988 to 2021. For the rainfall deviation map we downloaded rainfall data of 1987 to 1988, 1998 to 1999, 2009 to 2010 and 2020 to 2021 data from INDIA-WARIS official website. Then in GIS software we performed the IDW process over rainfall deviation data and created the rainfall deviation map.

DATA USED

Land Use Land Cover types and Changes of the Surroundings of Chilika Lake

This study conducted over Chilika and its adjoining areas provided us the first glimpse of land cover change over four decades. The basic land cover types we chose helps us to have an baseline understanding of the changes happening in the area which is important while trying to develop long-term studies of change detection in finer spatial and temporal resolution. The results from our analysis are shown below in figure no. 1 to 16 and the table shows the summarized result from the change detection. The following paragraphs provide a brief account of the results obtained. In the above maps Fig-2 (A to D) shows change in forest coverage between 1988 to 2021 over four decades. We classified the forest in three types of vegetation cover, one is Dense vegetation, second is Moderate vegetation and last one is Shrubs. Fig :2A is the 1988 image, Fig:2B is 1999, Fig-2C is 2010 and finally Fig-2D is the 2021 image. In those maps dense vegetation is shown in red colour, moderate vegetation is shown in orange colour and shrubs are shown in green colour. From those images we clearly see that in 1988 vegetation cover was low which gradually increased from 1988 to 2021.In 1988 dense vegetation cover in this area was 267153043.8 m2 which increased in 1999 and the area was 351280538.9 m2 but in 2010 because of population growth and fulfilling their demands deforestation occurred and the dense vegetation area decreased . To protect the forest, the social forestry programme and government forest departments take major initiatives and after 11 years the dense vegetation cover of the area has increased and the total 474062325.9 m2 area of the northern part of the study area is covered with dense vegetation. The moderate vegetation cover area was gradually increased from 1988 to 2021.

In 1988 it covered 352629206 m2 whereas in 2021 it covered 547051396.4 m2. It means the 194422190.4 m2 area is undergoing moderate vegetation cover over 33 years. And like others from 1988 to 2021 shrubs increased gradually. In 1988 shrubs covered 396727650.4 m2 but in 2021 it covered 656151275.3 m2. Overall, the forest coverage increased from 1988- 2021.Above bareland change is shown in Fig-3(A to D) over four decades between 1988 to 2021. We show the bareland area cover over an eleven year difference. Here, by Fig: 3A we show the 1988 bareland cover, Fig: 3B is for 1999 bareland cover, Fig: 3C for 2010 and last one is Fig: 3D for 2021 bareland cover. The bare land is mainly located between Chilika lake and the foothills area. From those maps we clearly see that in 1988 bare land cover in this area was high. The total area of bare land was 969565669.2 m2. But in 1999 it reduced slightly . In 1999 the area of bare land cover was 578623899.8 m2. It means it decreased to 390941769.4 m2 area. In eastern portion the bare land area decreased in 1999. In 2010 we again saw that the area of bare land cover increased slightly and it covered a total 1209472459 m2 area which was greater than 1988 bare land cover. But again after eleven years in 2021 it decreased majorly. In 2021 it covered a total area of 892996078.5 m2 . It means bare land extent lost in 2021 compared to 1988. And a total 76569590.77 m2 area was lost in four decades. From this analysis at last we clearly say that from 1988 to 2021 bare land extent decreased identically. The above map is created to show the change in Chilika Lake's water extent over decades. Here, the blue colour area is full of water content, red colour denotes emergent land and light yellow colour denotes sub-emergent land . Here, Fig: 4A is the 1988 image, Fig: 4B for 1999, Fig: 4C for 2010 and Fig: 4D for 2021. In our study area 52 river channels are present. Many of them carry sediment and deposit it in the lake for this reason lake depth is decreased gradually and also the natural mouth of the lake is silted. By the above map we clearly see how Chilika lake water cover area changed between 1988 to 2021. And from here we also see for depositional activity how emergent and submerging landforms developed and karka infestation increased in the emergent area day by day and also how the lake water retreated from the emergent land area. In 1988 Chilika Lake water cover area was 140360344.363916 m^2. Here we saw in the north eastern part or the head part of the lake, deposition work continuing and emergent and submerging landforms developing. In those emergent areas, a phragmites karka infestation is developing.

RESULTS

This study conducted near Chilika and its adjoining areas provided us the first glimpse of land cover change over four decades. The basic land cover types we chose helps us to have an baseline understanding of the changes happening in the area which is important while trying to develop long-term studies of change detection in finer

spatial and temporal resolution. This study also shows us how we can exploit the long-term Landsat archive for ecosystem studies at a reasonably fine spatial resolution which is otherwise not available from any of the other satellites data archives. The results from our study reveal that bareground and water land cover types decreased from 1988 to 2021 while shrub and forest types increased from 1988 to 2021. We have extended our study to Category wise land use land cover trend analyses just to identify and explore the reality Fig.5 (A to E). Trend line is the visual representation of pattern, direction and pace of change. A trend line is the practice of collecting information and attempting to sign a definite pattern. To identify the underlying land use land cover pattern over the past 33 year time span, we have adopted a trend analysis technique by applying a polynomial regression model of 3rd degree. Polynomial regression models have been adopted to analyze and identify the expected value of dependent variable `y' in terms of the value of independent variable `x'. The 3rd order polynomial regression model is used as its R-squared value is 1 in respect to the data set which represents the reliability of the represented trend line.

CONCLUSIONS

Within the framework of present discourse, for the detection of possible land use land cover changes, monitoring and evaluation in the Chilika Lake area using landsat TM and OLI data is easily realized. The image change detection conducted with GIS has been proven to obtain comprehensive information on the modifications and conversions of land use land covers as a result of spatio-temporal dynamics of natural and human activities. The result of present work indicates there has been important land use land cover change between 1988 to 2021 time-periods in the Chilika Lake area. Statistical analysis shows that the major changes have occurred in Vegetation cover, bare land and Chilika lake area categories. Here we declared other categories. From fig-5 (A to F) we clearly see the change trend. Here we clearly see that, vegetation cover change trend from 1988-2021 is upward. Vegetation cover means Dense vegetation, medium vegetation and shrub area cover is increasing from 1988 to 2021. On the other hand, if we discuss Chilika lake's freshwater area cover then we see that the trend line first moves downward then it is moved upward. It means from 1988 the area coverage decreased then it again increased slowly but compared to 1988 in 2021 water area coverage was low. The bare land cover trend line also decreased in 1999 then it increased rapidly from 1999 to 2010 then from 2010 to 2021 it decreased again. Overall, the bare land trend line nature is decreasing. For other categories from 1988 to 2021 the trend of change is downward. The trend line decreases rapidly. This change is directly or indirectly influenced by demographic changes and the overall change is remarkably influenced by the Chilika lake mouth shifting and silting. Our analysis provides us a baseline understanding of how the area has changed over the last three decades in terms of basic land cover types. This also helps us to focus on particular land cover types in future studies to help us understand how the changes in a particular land cover type might have an impact on available ecosystem goods and services of the area such as fishing and also its impacts on local climates. Thus we establish that usage of long-term satellite data records can help us to develop understanding of long-term changes in an area.

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11. Temperature Change Scenarios over the Chilika Lagoon of India during 1901-2100, Lalu Das, Monami Dutta, Jitendra Kumar Meher and Javed Akhter Chilika Lake – A Ramsar Site, Santosh Kumar Sarkar , Aparajita Bhattacharya , Asok Kumar Bhattacharya , Kamala Kanta Satpathy , Ajit Kumar Mohanty , Satya Panigrahi
12. Three decades of landcover change in Chilika and its Neighbourhood area using 30m Landsat data, , Santanu Goswami , Vivek G, S. B

Table: 1. Data Source

Data	Acquisition Date	Website
Landsat 5 TM(30m resolution)	07/01/1988	https://earthexplorer.usgs.gov
Landsat 5 TM(30m resolution)	07/12/1999	https://earthexplorer.usgs.gov
Landsat 5 TM(30m resolution)	25/04/2010	https://earthexplorer.usgs.gov
Landsat 8 OLI(30m resolution)	08/03/2021	https://earthexplorer.usgs.gov
Rainfall data	May 1987-88, May 1998-99, May 2009-10 and May 2020-21	https://indiawris.gov.in
Temperature data	1987-2020	power.larc.nasa.gov

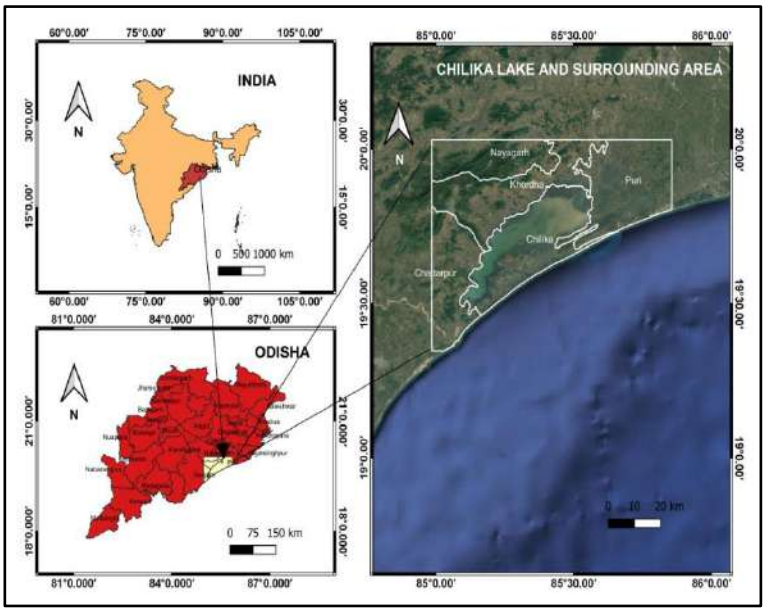
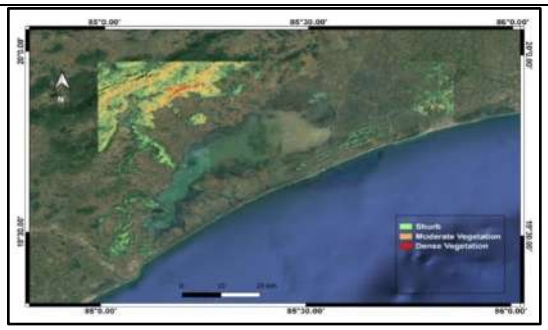
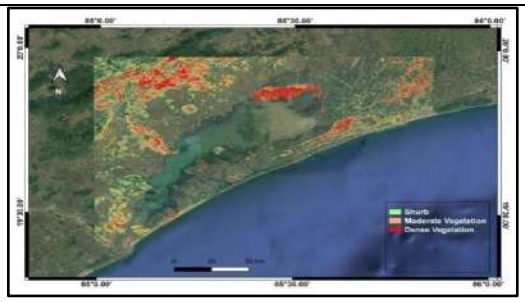


Fig 1: Study Area

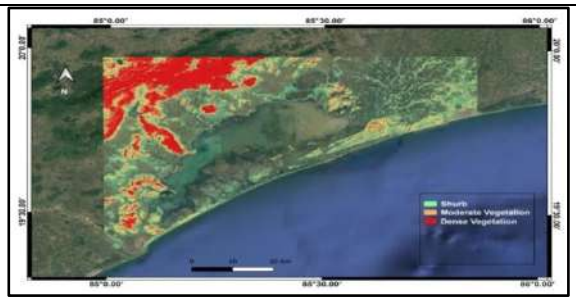


A (Year 1988)

B (Year 1999)



C (Year 2010)

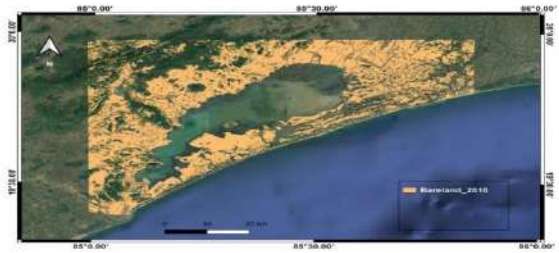


D (Year 2021)

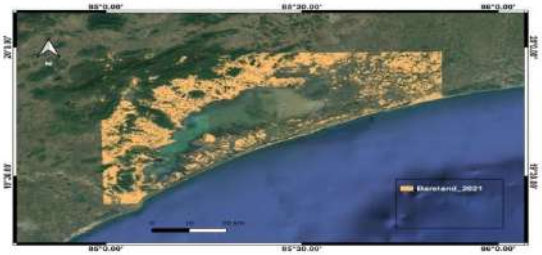
Fig 2: Vegetational Change Over Decades In Chilika Lake and Surrounding Area (Landsat Data)

A(Year 1988)

B (Year 1999)

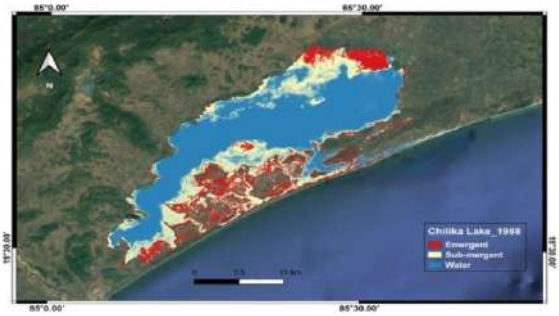


C (Year 2010)

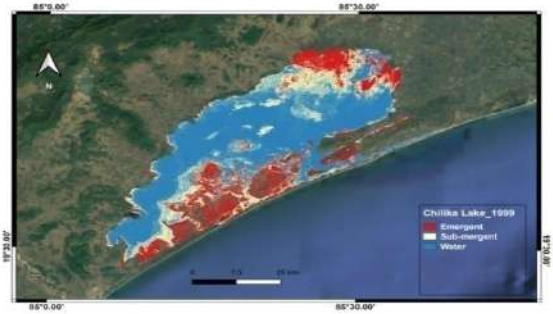


D (Year 2021)

Fig 3: Bare Land Change Over Decades In Chilika Lake and Surrounding Area (Landsat Data)



A (Year 1988)

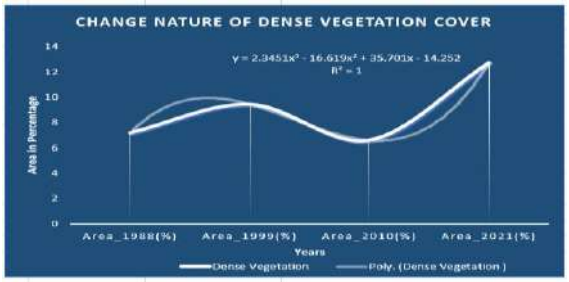


B (Year 1999)

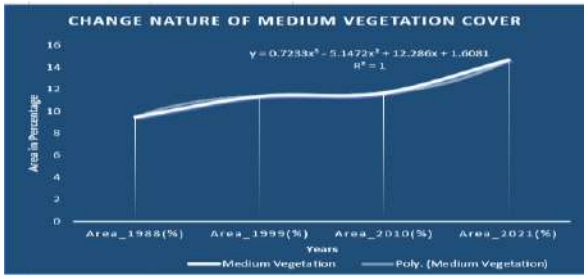
C (Year 2010)

D (Year 2021)

Fig 4: Chilika Lake's Emergent, Sub-emergent and Water Cover Area Change Over Decades

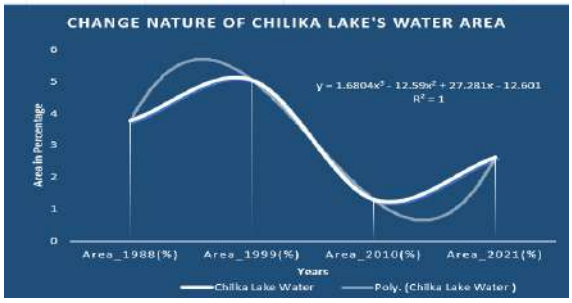


A- Change Nature of Dense Vegetation Cover

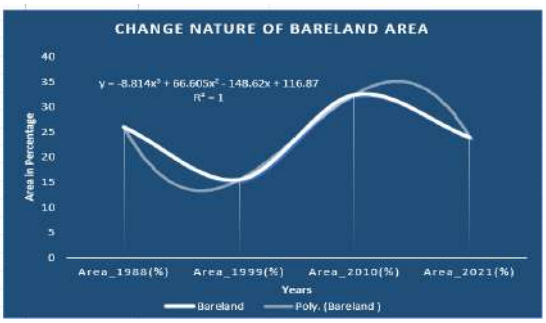


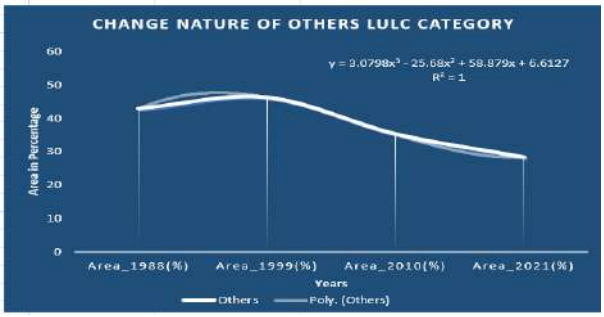
B- Change Nature of Medium Vegetation Cover

C- Change Nature of Shrub Cover Area



D- Change Nature of Chilika Lake's Water Area





E- Change Nature of Bareland Area	**F- Change Nature of Other LULC Categories**

Fig. 5: Changing Nature of LULC Categories

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RESEARCH ARTICLE

Occupational Health Risks of Kansaris in Bankura, West Bengal

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ABSTRACT

A significant portion of people is engrossed in brass and bell metal utensil making in the countryside areas of West Bengal in different ways either spontaneously or incidentally. This occupation is not devoid of health troubles. As the section of venture of brass and bell metal utensil manufacturing is basically bucolic and being disorganised, no attention has been given to the health risks of these artisans by legislators, public servants, developers and researchers. The purpose of the present investigation is to find out the occupational health risks of brass and bell metal utensil makers (kansaris) and to come up with some requisite proposals to mitigate these risks for achieving a more preferable standard of life for the craftsmen of rural Bengal. An intended systematized questionnaire, with leniency to tone with the regional outlook was applied to assemble the information from artisans. Total 64 artisans were selected for the study none of them were female. It divulged that in leading number of instances kansaris encounter hazards like respiratory problems, eye diseases, musculo-skeletal diseases, various visible health problems, skin diseases due to unhygienic occupational environments, work in uncomfortable posture for long time, using old tools and techniques, not using personal protective equipment and their negligence about personal health. So, kansaris require a significant degree of awareness through organised and persistent safety training for reducing their occupational health risks.

Keywords: Brass and bell metal, kansaris, rural, occupational environment, personal health, awareness

INTRODUCTION

Brass and bell metal utensil manufacturing is an ancient, native and conventional one. The uniqueness of this handicraft is that the technique of production is purely traditional and hereditary.[1]Numerous appliances for day-to-day uses, presents, devout customs and sculptures are made from these alloys. Brass and bell metal utensil manufacturing get going in Assam, West Bengal, Odisha and Uttar Pradesh give out regional in addition to

nationwide bazaar.[2]In West Bengal, brass and bell metal utensil manufacturing has been stationed mostly in two districts namely, Bankura and Murshidabad.[3]Brass is a mixture of copper and zinc with a ratio of 60% copper and 40% zinc, whereas bell metal is a mixture of copper and tin with a ratio of 78% copper and 22% tin .The traditional metal workers of West Bengal who work with copper and its alloys are known as kansaris. Kansaris are one of the most integral parts of our Bengali culture.[4] A significant portion of the total population of Bankura district is engaged in this sector. So this sector is vital and notable sector of pastoral financial resources in Bankura. Different types of works have to be done during brass and bell metal utensil making such as hammering, shaping, scraping, engraving, polishing, finishing etc. As this sector claims both physical energy and craftsmanship so mainly male family members are involved in this industry. The working environment of kansaris is not hygienic and they generally use conventional unsafe and unprotected equipment. So people working in this cottage industry suffer from various occupational health hazards. On the flip side, as this profession is bucolic and mostly unrecognised, very less concentration has been given to the wellbeing of these artisans by legislators, engineers and researchers.[5] Other problems of this industry are difficulties of availability of the basic materials from which products are made, lack of capital and fund, poor infrastructure, decrease in market demand of the products, conventional design and items, lack of safety training etc. Unskilled labours and craftsmen, who were connected with this profession over extended time are at present depart from this work for unsure superior alternative.[6]The main objectives of this present study are to find out the occupational health risks of kansaris in Bankura, West Bengal as well as to come up with some essential proposals to overcome these risks. So that they can achieve a more desirable standard of life for the artisans of rural Bengal.

MATERIAL AND METHODS

Study area: Three villages namely Mogra, Salbedia and Gogra associated with Bankura district of West Bengal, were selected as study area where a significant mass of kansaris are found. Sample: A sample of 8 brass metal and 8 bell metal production units i.e.,64 artisans were chosen for the investigation. Sampling technique: Stratified Random Sampling technique was used to conduct the study. Sources of data :Primary data: The current investigation mainly depends on the primary data source that was assembled through a field survey in the study area during the time span of July 2021 to October 2021 in the brass and bell metal production units(Picture 1 and Picture2).An intended systematized questionnaire, with leniency to tone with the regional outlook was applied to assemble the information from craftsmen. Prior to response, every query were minutely explained with the artisans. The eclectic artisans were interrogate to assemble facts according to the methodology adapted by Ganguly et. al., 2016.[7] During interrogation their working condition, socio-economic status, age, academic achievement, daily monthly earnings, obtainable government amenity and health condition were documented. The Snellen chart was used to measure visual acuity. The pulmonary function test of artisans was conducted by Peak Flow Meter according to the methodology adapted by Roy et. al., 2010.[8] Secondary data: Secondary data was taken from different research journals, publications of various agencies and websites.

RESULTS AND DISCUSSIONS

Age and gender of respondents : In the present study total 64 artisans were surveyed, none of them was female (Figure 1) showing that this cottage venture be in control of male artisans since it claims huge amount of physical strength and complicated crafting technique. The age status of respondents denotes that majority (34.375%) of the respondent are belonging to the generation range of 41-50 years while 29.687% among the total belongs to generation range of 31-40 years. This suggests that the efficiency of the artisans corresponding to the generation range of 41-50 years along with 31-40 years is relatively higher than other age groups. 17.187% of artisans are within the age cohort of 21-30 years, 12.5% belong to the age group of 51-60 years and the rest 6.25% are within the age cohort of 10-20 years. The proportion of artisans declines along winter of life which is obvious out of consideration of data appertaining generation range of 51-60 years. This may reveal that at older age most of the craftsmen are unable to do hard work. Very few artisans appertaining generation range of 10-20 years suggest that they are busy with their

education or not interested in such laborious work. Educational status of respondents: If we analyse academic background of the artisans , it is manifested (Figure 2) that majority (51.56%) of workers are belonging to standard V – VIII class, 21.87% belonging to standard IX – X, 17.187% belonging to standard XI and above. Only 9.375% studied up to class IV. The literacy rate among bell metal workers is 91.67% which is much higher in comparison to the national average (74.04%) (Census, 2010)[9]. Monthly income of respondents: Maximum number of respondents 46.87% (Table 1) are semiskilled worker and earn Rs. 10000 – 12000 per month. 29.68% of respondents are unskilled worker and earn Rs. 6000 – 8000 per month. A small portion (25%) of respondent earns Rs. 15000 – 16000 per month as they are skilled worker. If the remuneration of the workers rises to some extent then large number of persons will get absorbed in this cottage industry. Family structures of respondents: If we check out the construction of family of artisans (Figure 3) it will be clear that the majority (51.56%) is belonging to moderate sized family consists of 4-6 family members.35.94% are belonging to large sized family. A tiny segment (12.5%) of artisans associated with small family. So it may be concluded that the kansari profession involves almost all adult male members of the family. Occupational diseases of respondents: An work related illness is any persistent disorder contracted fundamentally in consequence of subjection to threat elements appearing from work activity[10]. It was assessed that in every single day almost 137 individuals pass away from job related diseases along with an extra 17 pass away from injuries all over the globe[11] ·Brass and bell metal workers experienced various types of occupational health problems.

- Respiratory problems of respondents: Kansaris are constantly exposed to significantly high levels of air pollution due to metallic vapour, dust and smoke. The pulmonary function test gives an understandable idea of the respiratory hazards (Figure 4) suffered by the craftsmen. Although majority (43.75%) of workers possess a normal lung function but 39.062% are suffering from shortness of breath and 17.187% are suffering from asthma. It has to be noted that problems increase in older workers as they are exposed to air pollution for very long time.
- Vision related problems of respondents: The eyes of the artisans are most affected by metallic vapour, dust and particles. Working long time under dim light put strain on the eyes and it may lead to watery eyes and poor eyesight. Eyesight (normal, myopia and hypermetropia) of artisans was assessed through eye testing utilizing the Snellen chart. Watery eyes is the most common symptom among artisans (37.5%) (Table 2) then majority (17.187%) is suffering from eye strain. 14.06% of workers are suffering from hypermetropia and 12.5% of craftsmen are suffering from myopia. Besides, a few old artisans are also suffering from cataract.
- Musculoskeletal problems of respondents: Kansaris work in uncomfortable position for long hours which led to various musculoskeletal problems. Recurrent disclosures to tremors along with jarring motions whilst running mechanical instrument[12-13]likewise generate numerous health problems. In aquaculture, damage related with musculature and bones take place as a result of frequent lifting or hand feeding, uplifting of hefty cages or bags of food, constant awkward positions at workplaces, and tractor use.[14-15]In the present study (Figure 5), most of the workers are suffering from pain in back (53.125%), knee (45.31%), leg (17.187%), joint (10.93%) and neck (4,687%). The repeated movement of the hand with the hammer leads to shoulder (25%), elbow (17.187%) and wrist (10.93%) aches. The problems increase with the increase in working years.
- Visible health problems of respondents: Among the workers numerous visible health problems are noticed. Calluses are formed on palms and fingers of majority (78.12%) of workers (Table 3). Callus is the outcome of repeated friction of the hammer with the hand. Burn spots are found on various body parts of 39.062% artisans. Carelessness for a movement during working near the fire may cause burn. 12.5% of workers develop cuts in hands due to handling of materials with sharp edges.
- Other health problems of respondents: Artisans have to carry out their work in unhealthy situations bring about numerous health hazards[16-17] Other health problems reported by artisans are skin disease (Itching, Acne) 32.81%, Paronychia (6.25%) due tolong exposure to metallic dust and worker's negligence about personal health (Figure 6).26.56% of artisans suffering from abdominal pain which may be connected to irregular eating habit. 1.56% of workers suffering from deafness which may be related to the noisy working environment .

Use of personal protective equipment (PPE) among respondents: Due to high temperature of the bhati (manual furnace) and hot metal the working environment is often very hot. So, most of the artisans go for working bare-chested and wearing nothing on the feet. In the present study, only 15.62% of craftsmen use towels on head (Figure

7). Very few of them use masks (4.64%), goggles (3.12%), caps (1.56%) and gloves (1.56%). According to them PPE is not comfortable to work with.

CONCLUSION

From the present study, it may be inferred that multiple types of occupational health hazards are found on a regular basis among brass and bell metal utensil makers. So there is a demand of significant degree of awareness and refinement by way of persistent safety training to minimize the health risks. In this situation, if numerous public services along with NGO's came ahead to repress the problems of these kansaris then after a short time many other impoverished people of rural Bengal take part in this industry to elevate their socio-economic status.

Recommendations

Educed from the understanding of the present investigation I suggest following essential proposals:

- Old tools and techniques should be replaced by modern tools and techniques.
- Work place should be maintained in the right way i.e., it must be well ventilated, spacious, adequately lighted.
- During working artisans should use PPE which diminish the probability of mishaps.
- To avoid paronychia, abdominal pain, skin diseases etc., craftsmen should attentive to keep their proper health and hygiene.
- Artisans should get organised and persistent safety training to make familiar with different types of prophylactic procedures to restrict hazards.
- Craftsmen should get systematic medical check-ups from government sectors.
- Workers should rotate their work and get rest periods i.e., short breaks during working period to avoid work in uncomfortable position for long hours.
- There is necessity of more elucidative research in concentration of advance spotting, prevention and superintendence of job associated hazards amongst kansaris.

ACKNOWLEDGEMENTS

I want to acknowledge Mr. Rajendra Prasad Mondal, Associate Professorin Zoology, Bankura Sammilani College, Bankura for his cordial help and support during various phases of the investigation. I acknowledge the help acquired from the kansaris of Mogra, Salbedia and Gogra village belonging to Bankura district, West Bengal, India for recording their experience.

Conflict of interest

Conflict of interest declared none.

Funding Sources

I have not received any fund to conduct this research work

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Table 1: Monthly income of respondents

Types of artisans	No. of artisans	Percentage (%)	Monthly income (in rupees)
Skilled	16	25%	15000 - 16000
Semiskilled	30	46.87%	10000 - 12000
Unskilled	19	29.68%	6000 - 8000

Table 2: Vision related problems of respondents

Types of vision related problems	No. of workers affected	Percentage (%)
Watering	24	37.5
Strain	11	17.187
Myopia	8	12.5
Hypermetropia	9	14.06
Cataract	2	3.125

Table 3: Visible health problems of the respondents:

Visible health problems	No. of artisans affected	Percentage (%)
Callus	50	78.12
Cuts	8	12.5
Burnspots	25	39.062

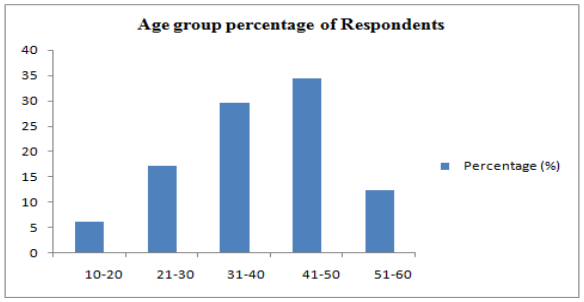

Figure 1: Age of respondents

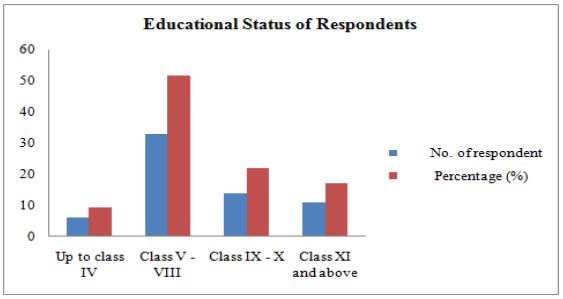

Figure 2: Educational status of respondents

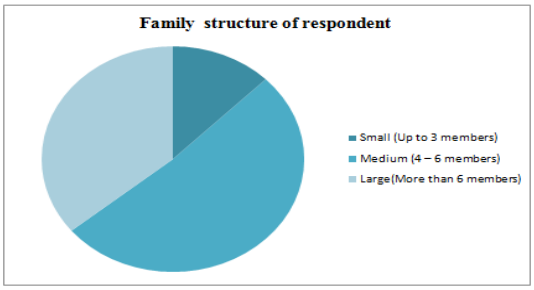

Figure 3: Family structure of respondents

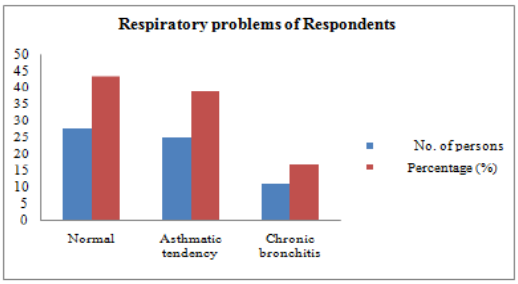

Figure 4: Respiratory problems of respondents

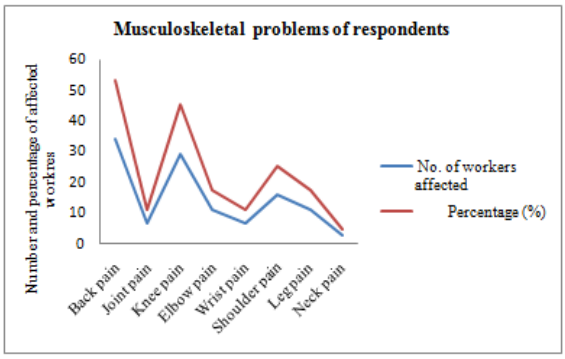

Figure 5: Musculoskeletal problems of respondents

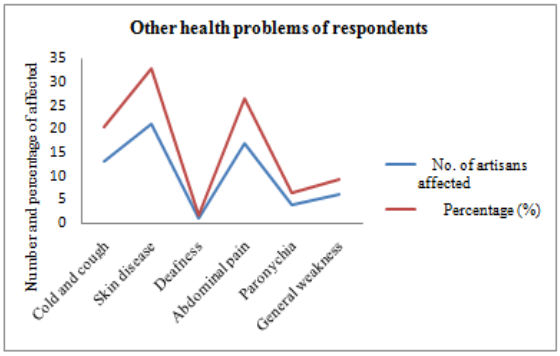

Figure 6: Other health problems of respondents

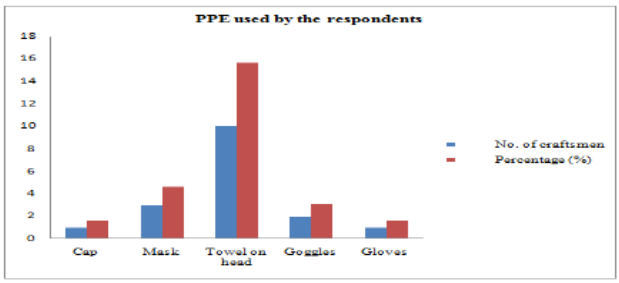

Figure 7: PPE used by respondents

Picture 1 Artisans busy in work

Picture 2: A craftsman engaged in finishing a plate (Kansa)

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REVIEW ARTICLE

Deep Vein Thrombosis in Pediatric Patients

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ABSTRACT

Deep vein thrombosis (DVT) is becoming more common in children due to developments in caring for critically ill children and those with chronic conditions. Central venous catheters, chronic medical problems, thrombophilia, and numerous drugs are also risk factors. Compression The most frequent approach for diagnosing DVT is Doppler ultrasonography, and patients usually present with discomfort and edoema in the afflicted limb. The most common treatment for children with DVT is anticoagulation via subcutaneous injection, and novel direct oral anticoagulants are now being studied. Although no proven prevention methods exist, clinical research are being conducted to address this need.

Keywords: anticoagulation therapy, coagulation thrombolytic, hematology hemostasis and thrombosis, thrombophilia, thrombosis, thrombotic disorders.

INTRODUCTION

A blood clot in the deep veins of the limbs, thoracoabdominal area, or brain sinuses is known as deep vein thrombosis (DVT).DVT was once thought to be a disorder that only affected adults. The rate of DVT in children is increasing as a result of improved paediatric care, particularly the increased use of central venous catheters (CVCs), as well as advances in clinical detection and radiographic techniques.[1]Patients with DVT have a higher fatality rate (up to 2 percent) [2] and morbidity, which includes the development of pulmonary embolus (PE) and post thrombotic syndrome (PTS) [3,4]. Because children have a longer life expectancy than adults, issues like morbidity and long-term effects become more important. A collaborative partnership called Children's Hospitals' Solutions for Patient Safety is working to avoid thrombosis in children (CHSPS). This organisation attempts to reduce thrombosis,

one of ten hospital-acquired (HA) diseases in children. Unfortunately, there are no effective preventative measures for venous thromboembolism (VTE), and rates of paediatric thrombosis are predicted to continue to rise. As a result, paediatric hematologists/oncologists must have a thorough awareness of the disease's prevalence, diagnosis, therapy, and prognosis. The goal of this study is to describe the risk factors for paediatric DVT, the presenting symptoms, diagnostic methods, treatment options, and long-term consequences.

EPIDEMIOLOGY

The vast majority of venous thromboembolism (VTE) incidents occur in children who are currently or have recently been hospitalised. HA-VTE, which comprises DVT and PE, has been continuously rising in prevalence. [1,5] According to the Kids' Inpatient Database, tertiary care centres have higher thrombosis rates than community hospitals.[5] The incidence of thrombosis in children follows a bimodal distribution, with the biggest peak occurring in children under the age of one month (14.5 per 10,000), and the second peak happening throughout puberty. [6,7] The neonatal group is a special group of sick, typically premature new-borns in whom CVCs are the leading cause of DVT. Non-HA DVT is more common in children under the age of 11 [8].

RISK FACTORS

Central venous catheters

The existence of a CVC is the most common risk factor for DVT in children. [2,6] DVT rates in children vary depending on the kind of CVC, the patient group, and the imaging technology used. This is most likely related to vascular damage induced by catheter insertion as well as irregular flow that happens when a catheter sits in the artery lumen. Non-tunneled (percutaneous) femoral and internal jugular CVCs, peripherally inserted central catheters (PICCs), and tunnelled CVCs, such as implanted subcutaneous ports or external catheters, are all examples of CVCs. Which type of CVC, insertion technique, or catheter material carries the highest risk of DVT formation is the subject of debate. DVT rates were lowest in patients who had PICCs and umbilical lines (rather than tunnelled lines) in a meta-analysis, with no difference in DVT rates between CVCs put in the upper and lower extremities. Tunneled lines, on the other hand, had the lowest rate of DVT (compared to non-tunneled and PICCs), according to a systematic review. The discrepancies shown above are most likely attributable to retrospective study designs, patient groups, and whether the outcome was symptomatic DVT or asymptomatic VTE, as measured by surveillance imaging.

Disease-related

Hospitalized children are at the highest risk of developing DVT due to a combination of risk factors, including the use of CVC, as well as immobility, inflammation, infection, surgery, and other causes. Acutely unwell children who are admitted to the hospital have a 3% greater risk of thrombosis for every additional day they are in the hospital.

The two chronic conditions with the highest risk of DVT are cancer and congenital heart disease (CHD). DVT has been documented in 7–34 percent of children with cancer. This is related to a variety of factors, including the disease itself, chemotherapeutic medications like steroids and asparaginase, the use of CVCs often, and infection. Thrombosis is also more common in older cancer patients and those with metastatic disease, according to studies. When patients with CHD are critically unwell, have a CVC in place, and have a single ventricle physiology, they are at the highest risk for thrombosis. These youngsters were discovered to have coagulation protein and blood flow irregularities, as well as difficulties with blood vessel wall integrity. 24 Furthermore, due to the low-flow state of the devices and their pro-thrombotic circuit material, children who require mechanical circulatory support are at an elevated risk of thrombosis. Other chronic conditions that cause DVT in children include gastrointestinal failure resulting to long-term total parenteral nutrition (TPN), inflammatory bowel disease (IBD), and dialysis-dependent children. Increased inflammatory cytokines, over expression of tissue factor, and poor fibrinolysis enhance the risk of thrombosis in patients with IBD. In addition, diseases that cause immobility, such as spina bifida and quadriplegia, have been linked to an increased risk of DVT due to venous stasis caused by inactivity.

Thrombophilia

Inherited and acquired thrombophilia have been related to a higher rate of thrombosis onset and recurrence in children and adults. The prothrombin G20210A and Factor V Leiden gene mutations are the most commonly tested thrombophilias, as are decreased proteins C, S, and antithrombin, raised lipoprotein (a), homocysteine, and factor VIII, and antiphospholipid antibodies. Routine thrombophilia testing is not recommended for adults, although studies have shown that testing can be beneficial in youngsters. The risk of thrombosis is low in a healthy child with known thrombophilia who does not have another risk factor for DVT. The additive risk of thrombophilia in children with a known risk factor, such as cancer, may be significant. Thrombophilia testing should be done in patients who have had an unprovoked VTE, those who have had recurrent VTE, and those who have a strong family history of thrombosis. The advantages of testing are less evident for other patients.

Medications

By modifying the nature of the coagulation and fibrinolytic systems, oral contraceptives have been shown to increase the risk of DVT, especially in the first year of medication. Increased prothrombin, factor VII, factor VIII, factor X, von Willebrand factor, and fibrinogen levels, as well as acquired resistance to activated protein C and increased fibrinolytic activity, are among the changes. Protein C, protein S, and antithrombin levels are all reduced as a result of the oestrogen component. Third-generation progestins have also been demonstrated in studies to increase the risk of thrombosis. Asparaginase, a common chemotherapeutic treatment for children with acute lymphoblastic leukaemia (ALL), inhibits the formation of coagulation proteins. This inhibition can raise the risk of thrombosis and bleeding, albeit the risk is usually more thrombotic because the anticoagulants, antithrombin, protein C, and protein S, are reduced. Corticosteroids, which are also part of the backbone in ALL therapy, are utilised in many other malignant and non-malignant disorders. Prednisone has been observed to raise factor VIII, von Willebrand factor, prothrombin, and antithrombin levels while decreasing fibrinogen and plasminogen levels. Activated prothrombin complex concentrates, some antipsychotics, all-trans-retinoic acid, many chemotherapeutic agents such as bevacizumab, doxorubicin, lenalidomide, thalidomide, as well as some immunosuppressive agents and thienopyridine derivatives have all been shown to increase the risk of thrombosis.

CLINICAL SYMPTOMS

Symptoms might take many different shapes. Swelling and pain, as well as warmth or redness of the affected limb's skin, are common symptoms of an extremity DVT. DVT in the superior vena cava (SVC) causes skin discoloration, pain, and swelling in the upper chest, neck, and head, resulting in SVC syndrome. Because symptoms are usually absent or vague, DVTs in the abdomen are more difficult to identify and diagnose. These symptoms are also caused by venous obstruction and result in swelling and discomfort in the affected area, which might manifest as left upper quadrant pain due to an engorged spleen in the case of splenic vein thrombosis, for example. Thrombosis of the portal vein is crucial to recognise because it can induce portal hypertension, splenomegaly, and upper gastrointestinal bleeding, which can be the presenting symptoms of a DVT. Renal vein thrombosis can result in both renal function and haematuria. Acute headache, vomiting, visual impairment, localised neurological impairment, and convulsions are all symptoms of cerebral sinus venous thrombosis. Catheter malfunction, which makes it difficult to infuse drugs or obtain blood samples, is a less obvious indication of catheter-related VTE. This symptom should prompt you to think about diagnostic imaging. DVT symptoms should be examined frequently in children with a CVC, especially those who are extremely unwell and hospitalised. Thrombocytopenia, which can be a marker of thrombosis in newborns and babies, is another modest symptom.

DIAGNOSIS

Imaging

The most frequent approach for diagnosing DVT is compression with Doppler ultrasonography. It identifies clots using three methods: direct viewing, compression, and colour Doppler flow. It's particularly helpful in the extremities, with >95 percent specificity in the proximal deep veins of the lower extremity in adults. In the upper extremities, caution is advised because sensitivity is reduced and a negative ultrasound does not rule out DVT. If an ultrasound is negative, venography was formerly the gold standard, and it is still very useful for

checking the extremities or thoracoabdominal area. Traditional venography has been superseded as the major imaging tool for the abdomen and chest by both magnetic resonance venography (MRV) and computed tomography venography in recent years. MRV is also the primary approach for detecting cerebral sinus venous thrombosis. Compression ultrasonography should be followed by confirmatory testing with contrast venography or MRV to discover anatomical anomalies of the upper extremity, such as Paget-Schroetter syndrome, or the lower extremity, such as May-Thurner syndrome.

Laboratory

DVT can be diagnosed with the help of a few laboratory tests. The D-dimer, a fibrinolysis marker, is frequently high in patients with thrombosis and may raise suspicion of DVT, although it cannot be utilised to diagnose DVT. The D-dimer has a high negative predictive value in adult patients and is recommended to be done before radiographic imaging in patients with clinical suspicion of DVT. The D-dimer was shown to be sensitive but only modestly specific in youngsters when used to predict thrombosis. Hepatic, portal, and renal vein thrombosis can cause abnormal renal and liver function testing.

TREATMENT

Anticoagulation is the preferred treatment for DVT in children unless there is a contraindication, such as an active or severe risk of bleeding. In patients with right to left shunting, anticoagulation is started to prevent thrombus expansion and embolization to the lungs or, in rare cases, the brain. Dosing, monitoring, and treatment length recommendations are mostly based on extrapolated data from the adult population. When treating neonates and infants, where the coagulation system is constantly changing and developing, this might be very problematic. The CHEST guidelines recommend anticoagulation treatment for individuals with VTE related to a recognised and resolved risk factor (such as a CVC) for 6 weeks to 3 months, and for children with an unresolved risk factor for 6–12 months, based on minimal data. Kids-DOTT is a randomised controlled study that will analyse the duration of anticoagulation therapy in children who have a new VTE due to a temporary or reversible risk factor. It will help to fill in the gaps in the paediatric treatment duration data.

Unfractionated heparin

Unfractionated heparin (UFH) is an anticoagulant that works by inactivating thrombin and activated factor X and potentiating the effects of antithrombin. It is given as a continuous intravenous infusion. The availability of a reversal medication, protamine, which can be employed in cases of bleeding, is one of the advantages. Increased dose is required in neonates due to low antithrombin levels, as well as in children under the age of one year due to increased heparin binding to non-antithrombin proteins. The activated partial thromboplastin time (aPTT) or the antifactor Xa assay can be used to monitor UFH, while studies have indicated that the antifactor Xa assay can take longer in the therapeutic range. Heparin-induced thrombocytopenia (HIT) is an uncommon but dangerous adverse effect caused by the formation of antibodies against platelet factor 4/heparin complex in 1–2% of infants exposed to heparin.45 Stopping all heparin products and starting a no heparin anticoagulant are part of the treatment.

Low molecular weight heparins

The anticoagulants most typically prescribed in children are the low molecular weight heparins (LMWHs), which include enoxaparin, dalteparin, tinzaparin, and nadroparin. They also have an anticoagulant action via antithrombin because they are heparin byproducts, but they are more focused on activated factor X. They've been demonstrated to be just as effective as UFH, but with less bleeding problems and HIT episodes. 46 LMWHs are usually administered twice daily as a subcutaneous injection to children, and the antifactor Xa assay is used to track their progress. Although trials in adults have indicated that once-daily LMWH administration is safe and effective for treating DVT, pharmacodynamic (PD) studies in children to support the feasibility of this dosing regimen have been mixed.47,48 Long-term LMWH use can cause osteopenia.

Parenteral direct thrombin inhibitors

Bivalirudin is a direct thrombin inhibitor (DTI) that prevents thrombin from producing fibrin and activating platelets. 50 It does not require antithrombin to be effective, unlike UFH and LMWH. Bivalirudin is a drug that is given as a continuous infusion to treat VTE and HIT. The aPTT can monitor levels, but according to a recent study, no drug monitoring is required. 51 Bivalirudin has been shown to help in early clot regression and resolution. 51 Argatroban, a continuous intravenous infusion DTI, is most commonly used in children who are suspected of having or are at risk of having HIT. 52Argatroban is metabolised by CYP3A4/5 in the liver, bivalirudin is partially metabolised in the kidneys, and the rest is broken down by intracellular proteolysis.53,54

Fondaparinux

Fondaparinux is a synthetic pentasaccharide that inhibits antithrombin-dependent factor Xa in a selective manner. 55 It can be used to treat VTE or HIT and is given as a once-daily subcutaneous injection. An antifactor Xa assay calibrated for fondaparinux is used for monitoring. Pediatric studies have shown efficacy and safety, as well as dose recommendations. 56

Vitamin K antagonists

Warfarin, one of the most extensively used vitamin K antagonists (VKAs), works by preventing the gamma carboxylation and consequently activation of vitamin K-dependent coagulation components II, VII, IX, and X. Warfarin can be taken once a day by mouth, however it has a lot of drug and food interactions. An international normalised ratio is used to assess the anticoagulant impact (INR). To avoid transitory hypercoagulability due to a decrease in proteins C and S, which are also vitamin K dependent, VKA must be started with another anticoagulant for at least 5 days and until a therapeutic INR is obtained.

Direct oral anticoagulants

The DTI, dabigatran, and the direct factor Xa inhibitors, rivaroxaban, edoxaban, and apixaban, are the two types of direct oral anticoagulants (DOACs). DOACs are licenced for the treatment, prevention, and prevention of stroke in individuals with nonvalvular atrial fibrillation. DOACs have been shown in studies to be as effective as VKAs, but with a considerably lower risk of bleeding. 57 DOACs, which have not yet been licenced for use in children under the age of 18, may allow youngsters to get anticoagulation without the need for injections or a battery of laboratory testing. Rivaroxaban and dabigatran have completed phase I and phase II pharmacokinetic and PD (PK/PD) investigations. Patients are still being enrolled in PK/PD studies for apixaban and edoxaban. 60,61 For rivaroxaban, edoxaban, and dabigatran, paediatric phase III trials comparing each DOAC to conventional anticoagulation (LMWH, fondaparinux, or VKAs) are open and recruiting patients. 62,63 Apixaban's safety and efficacy in preventing thrombosis in children with newly diagnosed ALL treated with asparaginase is being evaluated in a phase III randomised trial. 64 Orally, once or twice daily (depending on the medication), without any laboratory monitoring is required. Idarucizumab, a dabigatran reversal drug, has been licenced for use in adults, while andexanet, a factor Xa inhibitor reversal agent, is still being studied.

Thrombolysis

Thrombolysis is a more aggressive therapy option for the treatment of DVT. Thrombolysis can be administered systemically or through a catheter. Both high- and low-dose systemic thrombolysis regimens have been established in children, usually with concurrent anticoagulation and strict bleeding monitoring. 65,66 Catheter-directed thrombolysis entails inserting a large-bore catheter into the DVT-affected vein to allow for a gradual, direct infusion of the thrombolytic drug onto the thrombus. 67The major thrombolytic drug used for catheter-directed or systemic thrombolysis is tissue plasminogen activator (alteplase).Thrombolysis is often reserved in paediatrics for patients with severe limb-threatening or life-threatening thrombosis, although it is becoming more common in children, and it should be considered especially in children with nonprovoked big DVTs of the lower extremities. This method is more successful than anticoagulation in preventing PTS, according to small trials.68 In children with proximal leg DVT, a randomised controlled trial will be launched to compare the efficacy of catheter-directed thrombolysis followed by anticoagulation with anticoagulation alone.

LONG -TERM SEQUELAE

Recurrence and PTS are the two most common long-term consequences of DVT. Local recurrence or second thrombotic episodes in children range from 7% to 21%, depending on the patient's age, thrombophilia, and the inciting substance (such as a CVC). 2,6,30,69 PTS is caused by venous insufficiency caused by valvular injury, and it causes chronic discomfort and swelling, tingling, decreased exercise tolerance, and skin ulceration in severe cases. PTS has been discovered in up to 63 percent of children with DVT, particularly in those with numerous venous segments implicated and radiographic DVT resolution.70 71 Overuse-related DVTs in the upper extremity (sports, weight lifting, musical instrument playing) are more likely to develop PTS than CVC-related DVTs. 72 In upper limb DVT, a lack of DVT resolution or thrombosis extension is also a predictor of PTS. 72 Techniques to prevent PTS have not been proven in adult or paediatric patients, while compression stockings have been shown to help certain patients.

PREVENTION

In youngsters, prophylactic measures have yet to be proven successful. Mechanical and pharmacological prevention have been found to reduce the rate of VTE in adults, especially when used together. 73,74Mechanical prophylaxis refers to devices that apply pressure to improve lower limb venous circulation, such as intermittent pneumatic compression devices or graduated compression stockings. Anticoagulation is used in pharmacological prophylaxis, usually at lower levels than those used in treatment. In children, randomised controlled trials have been conducted to see if preventive UFH, LMWH, or warfarin can prevent VTE. 75–78 There was a difference in thrombosis rates between the therapy groups in one of the four trials, which included extremely ill trauma patients. Anticoagulation was not shown to be useful in the other three trials, which focused on preventing CVC-associated thrombosis. The risk of VTE must be evaluated in order to determine whether patients require VTE prevention. VTE risk prediction models for children have been developed, albeit the majority are single-institution studies or for specific patient populations. Mitchell et al. established a model to predict risk in children with ALL, while Raffini *et al.* produced a risk prediction model to avoid HA non-CVC-related VTE in teenagers. 79,80 Infection, prolonged hospitalisation, immobilisation, and the presence of a CVC were the biggest risk variables, according to three single-institution studies that were retrospectively verified. 17,18,81 More research is clearly needed in this area, and the Children's Hospital Acquired Thrombosis (CHAT) study is currently underway, with the goal of identifying risk factors, developing a risk prediction model, and determining thrombosis prevention strategies for hospitalised children at risk of thrombosis. 82,83

DISCUSSION

Clinicians caring for children with DVT must have a thorough awareness of the condition. All children having a CVC should be evaluated for DVT more frequently; line function and limb swelling should be assessed daily while hospitalised and at every ambulatory visit. In children without a CVC, every child who appears with a painful and swollen limb should be evaluated for DVT, especially in high-risk populations such as those with cancer or CHD, or a teenage girl who has recently started on oral contraception. Delays in diagnosis are typical in kids due to the low prevalence of DVT in the outpatient setting. Techniques for predicting and preventing DVT in children are also being investigated. 79,81–83 Children's thrombosis rates should, in theory, decrease once effective methods have been established. The paediatric thrombosis community is in desperate need of help right now, and all paediatric doctors, particularly haematologists and oncologists, should be aware of the shifting situation.

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RESEARCH ARTICLE

Effect of Different Level of NPK and Liquid Organic Fertilizers on DMP, Yield and NPK Uptake of Rice (*Oryza sativa* L.)

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ABSTRACT

A field experiment was conducted in the Experimental Farm, Department of Agronomy, Annamalai University during late *Kuruvai* season, 2018 in order to find out the effect of different organic and inorganic liquid fertilizer with different levels of NPK in rice (*Oryza sative* L.). The experiment was carried out in Randomized Block Design with three replication. The experiment consists with 12 treatments viz., T_1 - 100 % NPK + Humic acid granules @ 12.5 kg/ha, T_2 - T_1 + Panchagavya spray @ 3 % on 20, 35 and 50 DAT, T_3 - T_1 + Fish meal extract spray @ 3 % on 20, 35 and 50 DAT, T_4 - T_1 + Auxin Gold sea weed extract spray @ 0.35 % on 20, 35 and 50 DAT, T_5 - T_1 + Potassium nitrate spray @ 0.5 % on 20, 35 and 50 DAT, T_6 - T_1 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray on 20, 35 and 50 DAT, T_7 - 75 % NPK + Humic acid granules @ 12.5 kg/ha, T_8 – T_7 + Panchagavya spray @ 3 % on 20, 35 and 50 DAT, T_9 – T_7 + Fish meal extract spray @ 3 % on 20, 35 and 50 DAT, T_{10} – T_7 + Auxin Gold sea weed extract spray @ 0.35 % on 20, 35 and 50 DAT, T_{11} – T_7 + Potassium nitrate spray @ 0.5 % on 20, 35 and 50 DAT, T_{12} – T_7 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray on 20, 35 and 50 DAT. Among the different organic and inorganic fertilizers applied as foliar spray, application of 100% NPK + humic acid granules @ 12.5 kg/ha along with foliar application of Panchagavya @ 3% + Fish meal extract @ 3% + Auxin Gold sea weed extract @ 0.35% on 20, 35 and 50 DAT treatment performed better compared to other treatments and ranked first in terms of Dry Matter Production, yield and nutrient uptake. This treatment resulted in highest dry matter production (12286 kg/ha), grain yield (5660 kg/ha) and nutrient uptake.

Keywords: DMP, Grain yield, Panchagavya, fish meal extract, NPK uptake.

INTRODUCTION

Rice is the most important ancient crop cultivated in 117 countries and about 90 per cent of total rice is grown and consumed in Asia (Seema *et al.,* 2014). In Asia, more than 2 billion people are getting 60 to 70 per cent of their energy requirement from rice and derived products. In the world, rice is the second most widely consumed cereals next to wheat and it has occupied an area of 163.47 M ha with production and productivity of 501.56 MT and 4.58 t/ha respectively (USDA, 2019). Increasing trend of rice production during recent time is attributed to release of high yielding varieties and use of higher doses of fertilizer. But the use of higher dose of fertilizer (contain only N,P&K) and insufficient use of organics has created deficiencies of secondary and micro nutrients particularly of Zn and Fe. Apart from this the farmers have to use more and more fertilizer to obtain the same yield level as of previous years. Excess and imbalance use of chemical fertilizer has reduced the soil fertility status and reduced rice yield by 38 per cent (Singh *et al.,* 2001). Application of organic nutrient is of more appropriate because it contributes to the improvement and sustainability of natural resources and environment. A number of studies showed that crops in organic agriculture systems produce significantly higher yield than conventional agriculture (Pretty, 2000). Organic manures apart from NPK contain, small amount of trace elements especially boron, copper, iron, zinc and fair quantity of growth promoting substances. Foliar application is one of the method of fertilizer application in which fertilizers are applied in the form of solution on the foliage of the plant. But foliar application alone may not be sufficient to obtain optimum yield. Foliar applied nutrients entered the plant through stomata and by this method small quantity of nutrient are applied at critical stages of crop growth and are absorbed quickly and effectively.

MATERIALS AND METHODS

A field experiment was conducted at the Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar which is situated at 11°24' N latitude and 79°44' E longitude and an altitude of +5.79 m above MSL. The experiment was carried out in the Late *Kuruvai* season (July 2018) with testing rice variety of CO – 47. The experimental field is clayey loam in texture, low in available nitrogen, medium in available phosphorus, and high in available potassium. The experiment was laid out in Randomized Block Design with 12 treatments and replicated thrice. The experiment was T_1 - 100 % NPK + Humic acid granules @ 12.5 kg/ha, T_2 - T_1 + Panchagavya spray @ 3 % on 20, 35 and 50 DAT, T_3 - T_1 + Fish meal extract spray @ 3 % on 20, 35 and 50 DAT, T_4 - T_1 + Auxin Gold sea weed extract spray @ 0.35 % on 20, 35 and 50 DAT, T_5 - T_1 + Potassium nitrate spray @ 0.5 % on 20, 35 and 50 DAT, T_6 - T_1 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray on 20, 35 and 50 DAT, T_7 - 75 % NPK + Humic acid granules @ 12.5 kg/ha, T_8 – T_7 + Panchagavya spray @ 3 % on 20, 35 and 50 DAT, T_9 – T_7 + Fish meal extract spray @ 3 % on 20, 35 and 50 DAT, T_{10} – T_7 + Auxin Gold sea weed extract spray @ 0.35 % on 20, 35 and 50 DAT, T_{11} – T_7 + Potassium nitrate spray @ 0.5 % on 20, 35 and 50 DAT, T_{12} – T_7 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray on 20, 35 and 50 DAT. Panchagavya @ 3%, fish meal extract @ 3%, sea weed extract @ 0.35% and potassium nitrate @ 0.5% were sprayed oto the respective plots on 20, 35 and 50 DAT. Five plant samples were collected from each plot, shade dried and then oven dried at 70^0C for 72 hours. The dry matter production was computed treatments wise and expressed in kg/ha. These samples were grinded in willey mill and used for estimating NPK content by standard procedures. The nutrient uptake was calculated by multiplying the respective nutrient content with respective dry matter production and expressed in kg/ha.

RESULTS AND DISCUSSION

Among the treatments tested, T_6 - T_1 + Panchagavya + Fish meal extract + Auxin Gold sea weed extract spray on 20, 35 and 50 DAT ranked first compared to the rest of the treatments and resulted in higher dry matter production (12286 kg/ha). This is represented in Table 1. The probable reason is due to the application of NPK fertilizer plus growth hormones and nutrients present in the organic substances which might have stimulated the growth parameters. The addition of the humic acid can increase plant P and Ca content. The positive charge side of humic acid will absorb H_2PO_4 and the negative charge side absorbs Ca^{2+} to be transferred to the plant root. This result was

confirmed earlier by Mindari Wanti *et al*. (2018). The humic acid contains auxin which promotes catalytic activity, cell permeability and increases dry matter production. This finding is similar to that of Thakur *et al.* (2013). The humic acid acts as bio regulator, enters the plant during early stages of growth and additional sources of polyphenol that acts as respiratory catalyst and hence metabolism and growth of plant was high. Application of 25% less NPK compared to recommended NPK resulted in reduction in dry matter production upto 1786 kg/ha. However, this reduction was improved due to the foliar application of various liquid organic fertilizers from 9 to 417 kg/ha. Among the individual application of liquid organic fertilizers along with either 100% or 75% NPK, fish meal extract spray was found to be better. The highest grain yield (5660 kg/ha) was obtained in combined application of organic and inorganic fertilizers along with 100 per cent NPK + humic acid @ 12.5 kg/ha. That might be due to the highest number of productive tillers/m^2, number of grains/panicle and number of filled grains/panicle. Application of Panchagavya or fish meal extract or sea weed extract along with 100 or 75% NPK with humic acid granules 12.5 kg/ha increased the grain yield to the tune of 372,439, 327 and 387, 387, 398 kg/ha compared to 100 and 75% NPK + humic acid granules 12.5 kg/ha application. Yield increase was due to quick absorption and assimilation of more nitrogen, phosphorus, potassium and micro nutrients present in inorganic fertilizers and organic fertilizers. This leads to physiological and morphological character improvement and finally reflected in higher yield. Similar result was earlier reported by Priyanka *et al.* (2019). Similarly, 100 per cent NPK along with panchagavya spray resulting in higher grain yield was earlier reported byUpadhyay*et al.* (2019).

Nutrient uptake is a product of nutrient concentration and dry matter accumulation. Combined application of organic and inorganic fertilizers promoted nutrient utilization accounting for better NPK uptake of rice. Increased uptake of nutrient might be due to higher availability of nutrients from the soil reservoir and also from the added source of organic substances. The higher nutrient application might have increased nutrient content in soil solution, which reflected in terms of increased nutrient content in plants. Similar results were also observed by Parvez*et al.* (2008). Application of 25% less NPK compared to 100% recommended NPK along with humic acid granules 12.5 kg/ha resulted in reduced NPK uptake to the tune of 13, 6.4 and 12 kg/ha respectively. The combined application of Panchagavya + Fish meal extract + sea weed extract along with 100% or 75% + humic acid granules @ 12.5 kg/ha resulted with NPK values of 88.9, 33.8, 86.2 and 79.2, 25.7, 65.2 kg/ha respectively. NPK uptake of rice was significantly increased with combined application of 100 per cent NPK + organic fertilizers spray on 15, 30 and 50 DAT. Moreover, presence of macro (N,P,K and Ca) and micro (Zn,Fe,Cu,Ma) nutrients besides total reducing sugar (glucose) was observed in panchagavya. Chemolithotrops and autotropicnitrifires (ammonifer and nitrifier) present in panchagavya colonize in the leaves which increase the ammonia uptake and enhance the total N supply. The increased N uptake by the rice crop with humic acid application was attributed to better use efficiency of applied nitrogen fertilizer in the presence of humic acid. Humic acid application would have sustained the flow of ammonical nitrogen for longer period of time. When such N availability was coupled with enhanced activation of roots, it would have led to the better utilization of N by rice. Anion exchange phenomenon could be another reason for increasing P availability and higher P uptake by rice .The increased P uptake due to humic acid application was reported by PerumalPalanivel*et al.*. (2015). Rice being a monocot could have taken up more of K by virtue of its high root CEC. Application of sea weed extract triggered the growth of beneficial microbes and secretion of soil conducting substance by these microbes. Due to their microbial substance and soil improvement ability, it acts as soil conditioner.

CONCLUSION

Based on the field experimentation it may be concluded that, application of 100% NPK + humic acid granules @ 12.5 kg/ha along with foliar application of Panchagavya @ 3% + Fish meal extract @ 3% + Auxin Gold sea weed extract @ 0.35% on 20, 35 and 50 DAT performed better and ranked first in terms of Dry Matter Production, yield and nutrient uptake.

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Table 1. Effect of different level of NPK and liquid organic fertilizers on the Dry matter production, yield and nutrient uptake in rice.

Treatments	Dry matter production (kg/ha)	Grain Yield (kg/ha)	Nutrient Uptake (kg/ha)		
			N Uptake	P Uptake	K Uptake
100% NPK + Humic acid granules 12.5 kg/ha	10570	5030	81.2	27.1	70.1
T_1 + Panchagavya 3%	11507	5402	85.1	30.7	77.1
T_1 + Fish meal extract 3%	11812	5469	86.2	31.4	82.6
T_1 + Auxin Gold Sea weed extract 0.35%	11164	5357	84.7	29.2	76.7
T_1 +KNO_3 0.5%	10820	5296	82.7	27.6	73.2
T_1+T_2+T_3+T_4	12286	5660	88.9	33.8	86.2
75% NPK + Humic acid granules 12.5 kg/ha	8784	4240	68.2	20.7	58.1
T_7 + Panchagavya 3%	8893	4627	74.1	23.1	63.2
T_7 + Fish meal extract 3%	9201	4627	75.6	23.8	63.7

T_7 + Auxin Gold Sea weed extract 0.35%	8793	4638	72.2	21.9	61.9
T_7 +KNO_3 0.5%	8823	4326	70.6	21.8	60.2
T_7+T_8+T_9+T_{10}	9899	4931	79.2	25.7	65.2
S.Ed	188	90	1.4	0.5	1.3
CD (p=0.05)	391	186	2.9	1.0	2.7

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RESEARCH ARTICLE

A Production-Based Inventory Model with E-Waste Transformation to Sustainable Products

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ABSTRACT

The electronics industries of this present age are highly competent with advancing technology and manufacture smart electronic items employing intelligent and innovative production tools. The production rate of such items are increasing eventually to meet the dynamic demands of the customers of electronic era craving for novelty and this gets added to the growth of economy, but at the same time the electronic waste accumulation in manifolds upon completion of the production procedure is gaining more attention from environmental researchers and the electronic industries face challenges in handling E-waste in spite of stringent enforcement of E-waste rules across the globe. This paper proposes a few feasible E-waste minimizing strategies and a model for manufacturing inventory which integrates cost parameters of transforming E-waste to sustainable products is developed. The newly constructed inventory model comprises of E-waste conversion costs is an environmental sustainable model and it is a new venture in the area of waste management. This model involving analytical method of solution attainment is both cost effective and environmental efficient as it integrates both production and waste management and it will certainly set new benchmarks in sustainable inventory modeling.

Keywords: Production inventory model, electronic products, E-waste, sustainable products, waste management.

INTRODUCTION

The technical advancements along with its applications and the varied facets of digitalization have altogether made the present manufacturing trends expand and replace the traditional production phases of electronic industries. These industries are growing with smart and intelligent production systems and sub systems blended with advanced industry 4.0 production machinery. The production rate of electronic products is very high at recent times as the utility of electronic items have become a part and parcel of all our lives. The electronic products ease our routine activities but at the same time hurdle the environment when turned into waste. Electronic waste, often known as e-scrap, includes potentially dangerous compounds including mercury and lead, which may be harmful to humans and the environment if not properly handled. A statistics analysis from the United Nations Institute of Training and Research estimates that 50 million tonnes of electronic waste are generated yearly and it will get doubled in the coming years and at the same time only 20 % of disposed e-wastes are recycled. This disproportion between waste generation and recycling of waste is indeed a threat to both human and the environment. E-waste causes a lot of damage to the environment as it affects both air, water and land resources. The mismanagement of e-waste and absence of treatment before disposal are the root cause of several health disorders. The reaction of the electronic components with the environment damages the eco-system and questions the sustainability of all living organisms.

Primary circuit boards (PCB) made up of metals, polymers and ceramics account for a significant portion of e-waste. Ribeiro *et al.* have offered a brief overview of e-waste and its potential for chemical contamination [2]. The implications of e-waste on the environment were discussed by Nageswara Rao [9]. Sankhla *et al*. reviewed the negative consequences of e-waste on both people and the environment. The literature on e-waste impacts emphasized the dreadful impacts of both radioactive and non-radioactive elements on human health and environmental sustainability[10]. The generation of e-waste is inevitable but controllable by employing different strategies of e-waste management, especially by the right choice of waste disposal method. Saha *et al.* discussed about hazardous trash and the amount of e-waste produced at global level together with metal recovery procedures[4]. Sinha-Khetriwal *et al.* have analyzed the different disposal techniques of e-waste prevailing in many nations [3]. In addition to the strategy of e-waste disposal, the strategies pertaining to reuse of e-products after recycling and extraction of usable elements from e-waste using suitable methods are also proposed by environmental researchers. Abdelbasir *et al*. emphasized trash management methods, significance of processing e-waste, as well as the recycling methods for extracting metals and nonmetals [1]. Masuduzzaman et al suggested to use E-waste after processing as a fine or coarse aggregate in the production of durable concrete [7]. Ramanayaka *et al* explored the ways of deriving nanometals from e-waste [8]. Recovering is another strategy of managing e-waste along with the previously mentioned e-waste management techniques of extraction, recycling, and disposal. Dismantling, upgrading, and refining are the three basic phases in recovering elements from e-waste. Dismantling is performed manually, upgrading consists of shredding larger components to small pieces using separation processes. The final phase of refining is performed using hydrometallurgical, pyrometallurgical, electrometallurgical, and bio metallurgical processes. The process of recovering is critical in the transformation of e-waste into sustainable goods.

Inventory researchers have also contributed to e-waste management by developing inventory models focusing on various dimensions managing of e-waste and also on different facets of producing e-products such as warehouse management, price breaks, transportation. To mention a few, Yadav *et al* has developed an electronic component inventory model with the concept of ware housing and backordering using genetic algorithm [11]. Miah *et al* has proposed discounting supply chain inventory model[12]. Arindum Mukhopadhyay has formulated a transportation inventory model for electronic products [13]. Sasireka *et al* has developed an inventory model to mitigate e-waste. In the model proposed the costs associated with e-waste disposal was discussed [14]. Ritha *et al* constructed another e-waste associated inventory model encompassing the costs associated with disposing e-waste using the method of incineration [8]. To the best of our knowledge the inventory models in particular to e-waste management are only few in count and this has motivated the authors to frame inventory models with special focus on e-waste management. Also the existing inventory models concentrate only on the disposing strategy of e-waste management.

This paper develops an inventory model based on the model developed by Tapan [7]with the incorporation of recovering strategy of e-waste management in addition to waste disposal. The costs associated with the modalities of exercising this strategy are included in this proposed model. The recovery strategy performs a key role in creating sustainable products and it is a great breakthrough in the mission of environmental conservation. The earlier inventory models consisted of the concepts of disposal techniques and its related costs cum benefits but the newly developed blended model has included both the concepts of production and waste management and it will set new beginnings in the integrated research works on inventory modeling. The model will be more effective in practice as it convolutes two strategies for the management of e-waste like as waste transformation to sustainable products and waste disposal. The paper is organized in the following way, The model formulation is presented in section 2; Section 3 includes a numerical example as well as sensitivity analysis and the last section concludes the work.

MODEL DEVELOPMENT

An inventory model for e-waste mitigation is presented in this section incorporating the strategy of transforming e-waste to sustainable products based on the model development of Tapan with demand dependent on quality and price [7]. The inventory model developed by Tapan is extended to electronic product production model with the inclusion of e-waste management strategies. Let us consider a production system of electronic products that generates e-waste at the end of every time of production processes. The production system is keen on mitigating e-waste. In addition to e-waste disposal, the manufacturing system employs the waste-to-sustainable-products strategy, in this case let us assume that two sub products are produced from e-waste and the respective cost parameters are given below in Fig.1.

2.1 Notations and Assumptions

$\beta$is a portion of defective items.
$1-\beta$is portion of perfect items.
$C_s$is Set up Cost.
$C_u(q)$is unit cost. The formula is$f + gq$. Here f and q are constants.
$C_h(q)$is holding cost. The formula is $c + dq^2$. Here$c$ and d are positive constants.
$C_c$is the quality control cost.
C_a is the quality assurance cost.
k is Markup rate (k>0).
q is quality rate.
C_d is the Dismantling Cost
$C_g$is the Upgrading Cost
$C_r$is the Refining Cost
C_{s_1} is the sub product 1's set up cost.
C_{p_1} is the sub product 1's processing cost.
C_{s_2} is the sub product 2's set up cost.
C_{p_2} is the sub product 1's processing cost.
C_{ds} is the waste items disposal cost.
C_{amc} is the Machines AMC cost
C_v is the cost of remaining valuables.
C_p is the price gained from sub products.
In this approach, shortages are not permitted.
Time horizon is horizon less.
Production rate intervention with quality.
The Production rate lowers as the quality improves. It's written as

$$P(q) = P_1 + \frac{P_2}{q}, 0 < q_1 \leq q \leq 1. \quad (2.1)$$

The markup rate k as well as the quality rate q are both functions of $D(k,q)$.Demand function is declining in relation to k and a rising function in relation to q. People might doubt the quality of a low-cost goods due to their uncertainties. That is, demand will rise until a certain q, after which it will fall, making the demand function concave with respect to q. It denotes

$D_k < 0$ and$D_{kk} < 0$. $D(k,q)$must satisfy$(1-\beta)P(q) - D(k,q) > 0$ (2.2)

After time t=0, the production activity begins, and the goods' quality is assessed using automated quality inspection technology, with imperfect components isolated. The defective particulars are classified into distinct repairable areas based on their quality. At $t = t_1$, the production process comes to an end. Inventory built during $[0, t_1]$ decreases as demand increases. The inventory is refilled with the repaired goods at time $t = t_2$. Additionally, the generated e-waste is processed to create new sub products. A full cycle is defined by the time [0, T].Fig.1 depicts a typical graph of the system.

Credit Calculation

Amount manufactured in total during$[0, t_1] = P(q)t_1$; $P(q) = P_1 + \frac{P_2}{q}$,$0 < q_1 \le q \le 1$.

Quantity of defective goods produced overall= $\beta P(q)t_1$

Total number of perfect goods made= $(1 - \beta)\, P(q)t_1$

At time$t = t_1$ the stockpile amount is $S_1 = [(1 - \beta)\, P(q) - D\,(k,q)]t_1$

At $t = t_2$ the stockpile amount is$\beta P(q)t_1$

A cycle's total manufacturing cost is $C_u(q)P(q)t_1$.

The expressions of $t_2$and T are obtained from t_1:

$t_2 = t_1 + \left(\frac{S_1}{(D(k,q)}\right) = \left(\frac{(1-\beta)P(q)t_1}{D(k,q)}\right)$

$T = t_2 + \left(\frac{S_2}{(D(k,q)}\right) = \left(\frac{P(q)t_1}{D(k,q)}\right)$

Total Carrying Cost is$\frac{C_h(q)}{2}[S_1 t_2 + S_2(T - t_2)]$

$= \frac{C_h(q)}{2D(k,q)}[\{(1-\beta)^2 + \beta\}\{P(q)\}^2 - D(k,q)(1-\beta)P(q)]t_1^2$

Gross Revenue $is\ kC_u(q)\ \mathrm{P}(q)t_1$

The average net revenue (ANR) (t_1, k, q)per unit of time = $\frac{1}{\mathrm{T}}$ ×[Gross Revenue + Price of Remaining Valuables + Price gained from sub products– Set up Cost– Carrying Cost– Quality Control Cost–Quality Assurance Cost–Dismantling Cost –Upgrading Cost – Refining Cost–sub product 1's set up cost –sub product 1's processing cost –sub product 2's set up cost – sub product 1's processing cost – waste items disposal cost –Machines AMC Cost– Production Cost]

$= kC_u(q)D(k,q) + C_v(q) + C_p(q) - \frac{D(k,q)}{P(q)t_1}[C_s + C_c + C_a + C_d + C_g + C_r + C_{s1} + C_{p1} + C_{s2} + C_{p2} + C_{ds} + C_{amc} +$

$CuqP(q)t1$

$-\frac{C_h(q)t_1}{2}[\{(1-\beta)^2 + \beta\}P(q) - D(k,q)(1-\beta)]$ (2.3)

The Model's Solution

For a given q, the necessary conditions for the presence of a optimal value of ANR are

$\frac{\partial ANR}{\partial t_1} = 0$ (2.4)

$\frac{\partial ANR}{\partial k} = 0$ (2.5)

Using (2.4) we obtain the following.

$\frac{D(k,q)[C_s+C_c+C_a+C_d+C_g+C_r+C_{s1}+C_{p1}+C_{s2}+C_{p2}+C_{ds}+C_{amc}]}{P(q){t_1}^2} = \frac{C_h(q)}{2}[\{(1-\beta)^2 + \beta\}P(q) - D(k,q)(1-\beta)]$

$t_1 = \sqrt{\frac{2D(k,q)[C_s+C_c+C_a+C_d+C_g+C_r+C_{s1}+C_{p1}+C_{s2}+C_{p2}+C_{ds}+C_{amc}]}{C_h(q)P(q)[\{(1-\beta)^2+\beta\}P(q)-D(k,q)(1-\beta)]}}$ (2.6)

Taking second partial derivative of (2.3) with respect to t_1 we get

$$\frac{\partial ANR}{\partial t_1^2} = -\frac{2D(k,q)(C_s+C_c+C_a+C_d+C_g+C_r+C_{s1}+C_{p1}+C_{s2}+C_{p2}+C_{ds}+C_{amc})}{P(q){t_1}^3} < 0 \quad (2.7)$$

It implies that ANR is maximum at t_1 for given k and q.

Property 2.1 The square root expression undert_1 is positive.

Proof If $[\{[(1-\beta)^2]+\beta\}P(q) - D(k,q)(1-\beta)]$ is positive , the value of t_1 beneath square root takes a positive value.

$= [\beta P(q) + (1-\beta)[(1-\beta)P(q) - D(k,q)]$

As$[(1-\beta)P(q) - D(k,q)] > 0$, the square root expression under t_1 is positive. (proved).

Here are all the sufficient criteria for the occurrence of maximum

1) $\frac{\partial^2 ANR}{\partial t_1^2} < 0$ (2.8)

2) $\frac{\partial^2 ANR}{\partial k^2} < 0$ (2.9)

3) $\frac{\partial^2 ANR}{\partial t_1^2} \cdot \frac{\partial^2 ANR}{\partial k^2} - \left\{\frac{\partial^2 ANR}{\partial t_1 \partial k}\right\}^2 > 0$ (2.10)

We have already proved the first condition.

(2.3) implies $C_u(q)D(k,q) + [kC_u(q) - A(t_1) + B(t_1)]D_k = 0$ for which

$$A(t_1) = \left[\frac{C_s+C_c+C_a+C_d+C_g+C_r+C_{s1}+C_{p1}+C_{s2}+C_{p2}+C_{ds}+C_{amc}}{P(q)t_1} + C_u(q)\right]$$

$$B(t_1) = \frac{C_h(q)(1-\beta)t_1}{2}$$

$A(t_1), B(t_1) > 0$ (2.11)

Taking second partial derivative of (2.3) with respect to k we get

$$\frac{\partial^2 ANR}{\partial k^2} = 2C_u(q)D_k(k,q) + [kC_u(q) - A(t_1) + B(t_1)]D_{kk}$$

$D(k,q)\cdot A(t_1)$is the cost of Set up Cost , Production Cost, Carrying Cost, Quality Control Cost, Quality Assurance Cost, Dismantling Cost, Upgrading Cost, Refining Cost, sub product 1's set up cost , sub product 1's processing cost , sub product 2's set up cost , sub product 1's processing cost , waste items disposal cost, Machines AMC cost and $kC_u(q)D(k,q)$ denotes selling price per unit. In any firm, $kC_u(q)D(k,q) > D(k,q)A(t_1)$therefore $kC_u(q) > A(t_1)$.

Hence, $\frac{\partial^2 ANR}{\partial k^2} = 2C_u(q)D_k(k,q) + [kC_u(q) - A(t_1) + B(t_1)]D_{kk} < 0$

We have proved the second condition. It is challenging to prove (2.10) analytically, but with the use of model parameters, this could be done and the ideal ANR value can be determined.

The Optimum Production Quantity $Q^* = P(q)t_1$

$$= \sqrt{\frac{2D(k,q)[C_s+C_c+C_a+C_d+C_g+C_r+C_{s1}+C_{p1}+C_{s2}+C_{p2}+C_{ds}+C_{amc}]}{C_h(q)[\{(1-\beta)^2+\beta\}-D(k,q)(1-\beta)/P(q)]}} \quad (2.12)$$

Algorithm

The following is an algorithm to solve the problem.

Step 1: Choose a demand pattern. Model parameters must be provided.

Step 2: Choose a q increment, say x. Let x be 0.1 otherwise 0.01. Create a counter y and set y=1.

Step 3: Let$q = q_1$and k=1.

Step 4: Using (2.6) find the value of t_1.

Step 5: Using (2.11) find the value of k.

Step 6: Steps 3,4 and 5 must be repeated until t_1 and k are consistent.

Step 7: Find ANR using (2.3). Enable this value to be ANR(y).

Step 8: $q = q_1 + x$

Step 9: Go to the following step if q is greater than 1. If not, enter y=y+1 and move on to Step 3.

Step 10: From all the ANR(y) find ANR(y) for which ANR is maximum. The model's solution will be determined by q, k and t_1.

Step 11: Stop.

NUMERICAL EXAMPLE

Consider a linear demand patternu+vr-wk with the model parameter values shown in Table 1.
Then the Optimum result is
t_1^*= 0.250875
k = 2.007669
ANR = 1819.676
Optimum Production Quantity ≈ 83

Sensitivity Analysis

Table 2. which follows, shows the annual net revenue numbers for various model parameter values. The parameters u, v, w, k and t_1 are varied by +15% to -15%. Following are some observations:

1. The demand parameter u is very sensitive especially for higher values. A positive change in u will increase the value of ANR.
2. The demand parameter v is less sensitive. A positive change in v will only increase the value of ANR.
3. The demand parameter w is very sensitive. A negative change in w will increase the ANR.
4. The decision variable k is very less sensitive. Any small change in k will lower the ANR.
5. The decision variable $t_1$is more sensitive especially for higher values. A positive change in t_1 will increase the value of ANR.

To acquire the best ANR value, the demand parameter and decision parameters must be accurately assessed. Fig. 2. provides a visual representation of the sensitivity analysis's findings.

CONCLUSION

This paper proposes an environmentally more responsible inventory model encompassing e-waste management strategies. The newly developed model is an extension of the existing model focusing on the production of electronic items and handling of e-waste. The key characteristics of the inventory model are the inclusion of the expenses related to the joint strategies of waste transformation into sustainable goods and e-waste disposal. The sensitivity analysis has also laid a clear picture of the parameters influencing the annual net revenue. The proposed model shall be extended further by employing other strategies of e-waste management and the deterministic model shall be characterized using fuzzy parametric values.

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Table 1: Input data of the Model

Parameter	Cs	Cc	Ca	Cd	Cg	Cr	Cs_1	Cp_1	Cs_2	Cp_2	Cds	$Camc$	u
Value	100	50	50	20	30	30	70	30	50	20	20	60	200

Parameter	v	w	P_1	P_2	c	d	β	f	g	Cv	Cq
Value	30	80	300	50	6	8	0.2	13	20	300	50

Table.2 Values of ANR

Parameter	**% Change**	**ANR**	**%CHANGE IN ANR**
u	-15%	1062.87	-41.59015121
	-10%	1292.345	-28.97938974
	-5%	1544.699	-15.11131652
	5%	2117.079	16.34373372
	10%	2436.746	33.91098196
	15%	2778.549	52.69471049
v	-15%	1693.151	-6.953160892
	-10%	1734.82	-4.663247743
	-5%	1776.996	-2.345472491
	5%	1862.861	2.373224684
	10%	1906.55	4.774146606
	15%	1950.743	7.202765767
w	-15%	2655.235	45.91800958
	-10%	2340.718	28.63377876
	-5%	2046.069	12.44139067
	5%	1603.002	-11.90728459
	10%	1410.341	-22.49493866
	15%	1238.639	-31.93079427

k	-15%	1597.334	-12.21876862
	-10%	1721.129	-5.415634432
	-5%	1795.117	-1.349635869
	5%	1795.307	-1.33919445
	10%	1772.679	-2.582712527
	15%	1602.729	-11.92228726
t_1	-15%	1065.657	-41.437
	-10%	1287.115	-29.2668
	-5%	1538.353	-15.4601
	5%	2131.086	17.11349
	10%	2472.217	35.86029
	15%	2842.465	56.2072

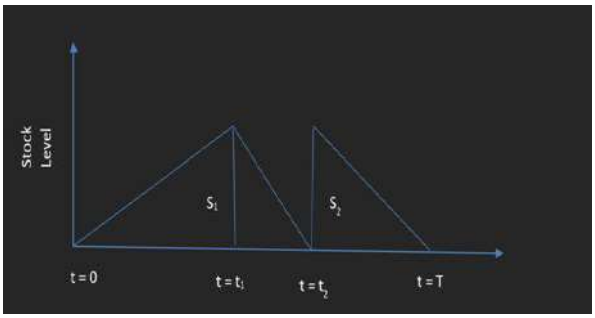

Figure 1: Graphical representation of E-waste

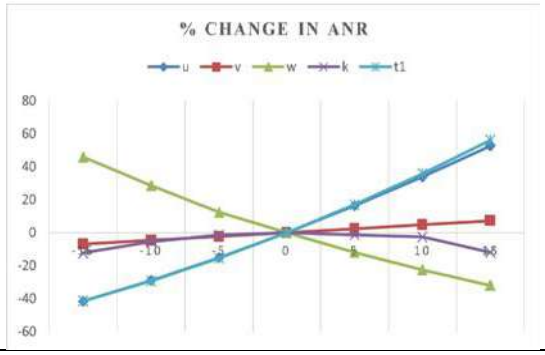

Fig 2: Picture representing the % change in ANR

RESEARCH ARTICLE

Nature: An Atheneum of Anti-Neuraminidase Biomolecules

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ABSTRACT

Most flu virus has neuraminidase (NA) and haemagglutinin (HA) antigenic glycoproteins on its surface. Though NA and HA have different properties, but their proper coordinated activity easily helps the virus to enter the host body. Because of the critical residues in the active site, NA receptor has become the center of attraction for antiviral treatment. While many new molecules have been approved by FDA but severe side effects and cases of drug resistance are the major problems in present situation. The Increasing threat of viral outbreaks has remained a serious concern for public health. Sudden alterations in NA susceptibility exposed the emergency requirement of new molecules as NA inhibitors. Nature provides a plethora of biomolecules with high diversity, which can facilitate target specific drug development in the fight against this disease. Although there are many reported molecules from plants, compounds of potent activity are taken in to consideration. This present review is an update on NA enzyme, its crystalline structure, function, mechanism and its inhibition by molecules present in nature.

Keywords: Neuraminidase, glycoproteins, target, inhibitors, drug discovery, natural molecules.

INTRODUCTION

These swift outbreaks and mutated viral strains, that are resistant to most of the available drugs are an alarming worry and this indicates the need of novel antiviral therapeutics. Stringent strategy, surveillance and development of novel lead structure have become essential to tackle the problems of presently exhisting antiviral drugs (like side effects, resistance and bioavailability) are fields of enormous research. In this quest for natural potent antiviral, natural products can play an important role as they contain wide range of bioactive molecules for drug design and development. In the past, scientists have extensively focused on the natural sources and examined the isolated molecules for their potency against viral infections. The main objective of present review is to expose specifically the natural compounds that inhibit the glycoprotein NA which is present on the viral surface, and to focus on the future benefits. This compilation of literature as a review will provide important information for further research in this field. Influenza A virus has the capacity to infect humans and other animals, on the other hand influenza B virus are reported to circulate only among humans producing outbreak of seasonal infections. Influenza C viruses are found to infect both humans and pets likepigs, being less severe among the all other strains, while influenza D affect cattle's and up to now not known to spread in humans [1,2] . Haemagglutinin and neuraminidase glycoproteins are present on the surface of Influenza virus. Haemagglutinin is a fusion protein which facilitates viral entry by joining to the terminal sialic acid found on the surface of the host cell. Generally neuraminidase binds to sialic acid and breaks the alpha ketosidic bond joining sialic acid and the sugar residue beside it on the host cell glycoproteins as well as the virus progeny [3,4]. Neuraminidases, also known as sialidases, catalyze the hydrolysis of terminal sialic acid residues from the newly formed virions and from the host cell receptors. This helps in the mobility of virus particles through the respiratory tract mucus and in the release of virion particles from the infected cell [5]. Neuraminidase looks like a mushroom shaped extension on the structure of the influenza virus. It has a head containing 4 coplanar, roughly spherical subunits, and a lipophilic area that is encapsulated inside the interior of the virus membrane. It consists of a single polypeptide chain that is oppositely directed to the HA antigen. The configuration of the polypeptide is a single chain of 6 polar amino acids, followed by hydrophilic, variable amino acids. [6].

Neuraminidase inhibitors in Phenolic compounds

Most of the research among phytochemicals focuses extensively on the applications of phenolic moieties (flavonoids) that are widely spread in natural sources. Flavonoids are known to be a different set of metabolites posessing less molecular weight and widely reported for their better bioactivity. Flavonoids are generally classified into 10 subclasses, i.e. aurones, biflavonoids, catechins, chalcones, flavanones, flavanonols, flavans, flavones, flavonoles, and isoflavones [7]. Important reported biomolecules are hereby collected in this review. Prenylated flavonoids were studied by Grienke U et al., for their potency against influenza virus NA . In this research seven active molecules were isolated from the plant *Morus alba* and tested their potency (IC_{50} of sanggenon C (11.3 ± 1.27 μM), sanggenon G (2.91 ± 0.65 μM), sanggenol A (6.22 ± 1.18 μM) are reported). All compounds produced activity against both NA and Streptococcus pneumonia when compared to standard oseltamivir [8] . Phytochemical analysis of *S. plebeia* facilitated in the identification of 14 molecules including two new compounds (1-2), monoterpenes having phydroxybenzoylated glucose moiety. As per the author's information, plebeioside A is the first natural monoterpenoid having a camphene skeleton. Among all the molecules, compounds 6, 10, 13, and 14 were isolated from this plant species for the first time. These molecules were analysed for their activities against Influenza A (H1N1) NA. It was reported that compounds 5 (19.83 ± 2.28 μM), 7 (11.18 ± 1.73 μM), 9 (16.65 ± 0.91 μM), and 11 (17.96 ± 2.38 μM) produced impressive results when compared to standard Oseltamivir. It was observed that Compounds5, 7, and 11 contains flavone skeletons while compound 9 was found to have an esterified phenyl propanoid ring system [9]. E Walther *et al*., evaluated the two neuraminidase inhibiting natural compounds, the diarylheptanoid katsumadain A and the isoprenylated flavone artocarpin. Various enzymatic assays were performed to find the inhibitory potency (IC_{50} value) of these molecules against pneumococcal NAs. Enzyme kinetics was studied to explain the mechanism of actions. Artocarpin exhibited a mixed type inhibitions and the Ki value was found to be 9.70 μM. [10]. Nur Kusaira *et al.,* reported a set of tropical plants possesing potent inhibitory activities against neuraminidase. Leaves, roots, and fruits of these plants (*G. mangostana*, *E. longifolia*, *T. divaricata*, *B. javanica*,

and *M. charantia*) were used in different extraction methods, followed by biological assay. Extracts of the above five plant produced moderate neuraminidase activity. It was reported that *G. mangostana* produced the maximum inhibitions (82.95% at 250μg/mL). Isolation of Rubraxanthone, α-mangostin, and garcinone C, having IC_{50} values in a range from 89 to 95 μM, were noteworthy [11]. Ha TK *et al.,* reported the potential neuraminidase (NA) inhibitory activity of ethanolic extract of *Cleistocalyx operculatus* leaves. Bioassay analysis helped in the identification of two novel acetophenones (compound 1-2) and one novel flavanone (compound 3). Elucidation of the isolated compounds was done by using various spectroscopic methods.

It was found that Compounds 6 and 8 produced potent enzymatic inhibition against different NAs from different viruses (IC_{50} values in a range of 5.07 ± 0.94 μM to 9.34 ± 2.52 μM)[12]. Nguyen NH *et al.,* reported the isolation of acetophenones containing spiroketal-hexofuranoside ring system, one di-C-glycosidic acetophenone and two benzopyrans, from the leaves of *Melicope pteleifolia*. 1D, 2D-NMR and HRESIMS spectroscopic methods were involved in the structure elucidation of the isolated compounds. All the compounds were tested for their NA inhibitory activities against various virus strains. From all the compounds, it was reported that tamarixetin 3-robinobioside produced impressive enzymatic inhibition (IC_{50} 24.93 ± 3.46 μM) [13]. Chen R A *et al.,* investigated the callus cultures of *Dysosma versipellis* which facilitated the identification of five new flavonol dimers, dysoverines A-E (1-5), along with another set of 12 compounds (compounds 6-17). Extensive spectroscopic data analyses were performed to characterize the structure of isolated compounds. Compounds 1-17 produced moderate NA inhibitory potencies (IC_{50} values of 37.0-93.9 μM). Compound 17 exhibited most potency (37.0 μM) than all compared when compared to standard Zanamivir [14]. Li P *et al.,* reported that punicalagin present in plants exhibited excellent anti-influenza potency, The IC_{50} value was very low in tissue culture. Various assays like Single cycle replication, neuraminidase inhibition, and virus yield reduction was performed to find the mechanism of action of the above molecule. It was observed that the above compound inhibited viral NA activity and thus helped in blocking the release of virus. It was also observed that punicalagin inhibited replications of various viral strains; resulting in the conclusion that punicalagin has wide spectrum antiviral activity against both IAV and IBV strains [15].

CONCLUSION

Medicinal plants are a source of biologically potent photochemical with desired therapeutic activities that over time have been studied and used by diverse groups of people for treating various symptoms and diseases. Medicinal products from nature sources have attracted the interests of researchers around the globe since many decades because of their minimum side effects and positive effects on human health. It has been reported by W.H.O that nearly 75-80% of the world's developing population trusts on traditional medicines for their primary health care needs. In this review we have reported the structures of various phytochemicals, source and their potencies as neuraminidase inhibitors. A wide range of various species still remains untouched and unused in terms of their phytochemicals as well as their pharmacology and this is the reason of research gap for future investigations. Further investigations of different species of plants and their phytochemical constituents are needed to design, discover and formulate safe medicines for human use.

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the above manuscript.

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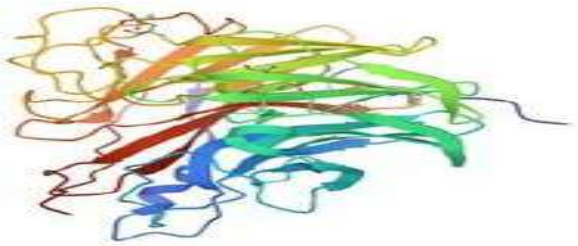

Figure 1: Structure of viral neuraminidase (PDB DOI: 10.2210/pdb1NSD/pdb)

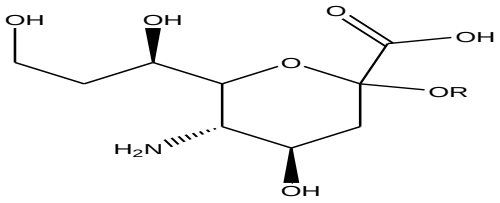

Figure 2: Structure of sialic acid

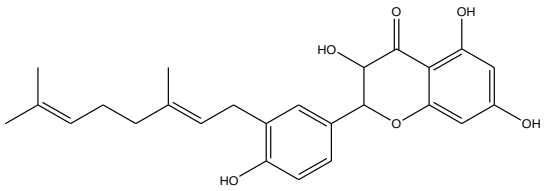

Figure 3: Structure of sanggenol

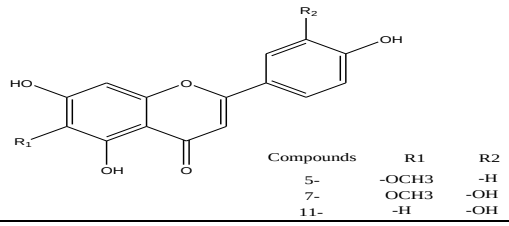

Figure 4: Structure of isolated compounds from *S. plebeia*

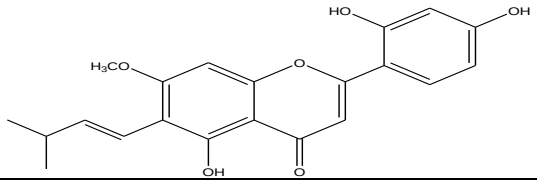

Figure 5: Structure of artocarpin

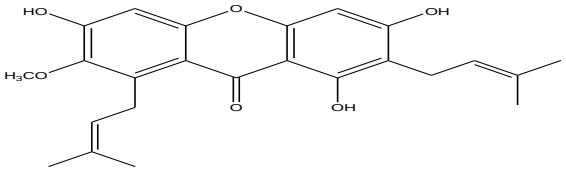

Figure 6: Structure of α-mangostin

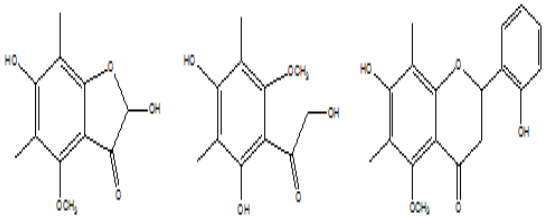

Figure 7: Structure of compound 1, 2 and 3

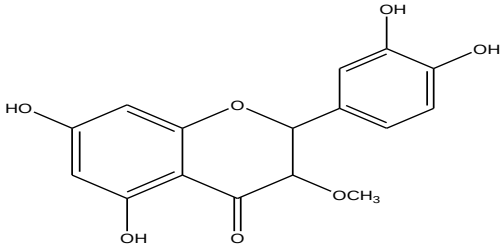

Figure 8: Structure of compound 17

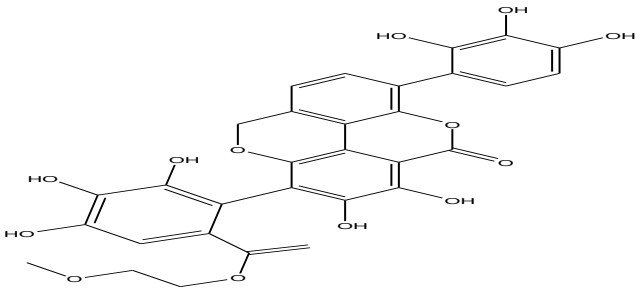

Figure 9: Structure of punicalagin

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RESEARCH ARTICLE

Effect of Organic Nutrients on Nutrient Uptake and Post Harvest Soil Nutrient Status in Cucumber (*Cucumis sativus* L.)

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ABSTRACT

Field experiment was conducted to study the influence of organic nutrients on nutrient uptake and post harvest soil nutrient status of major nutrients in cucumber (*Cucumis sativus L.*) during 2019 at Koneripalayam village in Perambalur district, Tamil Nadu. The popular variety Green Long suitable for this region was used for the present study. The experiment was carried out during two seasons *viz.*, Season I (February-May) and Season II (July-October) in Randomized block design with fifteen treatments and three replications. The results of the experiment revealed that the highest nitrogen (148.87 kg ha^{-1} in first season and 161.27 kg ha^{-1} in second season), phosphorus (33.90 kg ha^{-1} in first season and 36.31 kg ha^{-1} in second season) and potassium uptake (156.21 kg ha^{-1} in first season and 169.14 kg ha^{-1} in second season) was observed in the treatment T_{15} which received EM @ 1 t ha^{-1} + CBF @ 2 kg ha^{-1} + Se (20µg L^{-1}) as foliar application. With regard to the post harvest nutrient status *viz.*, nitrogen (174.61 kg ha^{-1} in first season and 181.20 kg ha^{-1} in second season), phosphorus (17.45 kg ha^{-1} in first season and 20.61 kg ha^{-1} in second season) and potassium (298.14 kg ha^{-1} in first season and 319.05 kg ha^{-1} in second season) status was recorded to the highest in the treatment which received FYM @ 25 t ha^{-1} + CBF @ 2 kg ha^{-1} + Se (20µg) as soil application. Among all the organic manures, EM was found to be the best organic manure in increasing the nutrient uptake and farmyard manure was proved to be superior in respect of post harvest soil nutrient status combined with consortium biofertilizer and selenium application.

Keywords: Organic manures, nutrient uptake, post harvest soil nutrient status, cucumber

INTRODUCTION

The concept of eco-friendly organic agriculture is gaining popularity as it helps in reducing the loss of nutrients, increases the availability of nutrient uptake leading to sustainable production of quality vegetables. Organic farming provides safer food without deteriorating the environment and it has positive influence on soil texture and water holding capacity. FYM, vermicompost, enriched manure and biofertilizers are gaining importance for obtaining higher yield and quality. Farmyard manure and vermicompost are bulky organic material, releases the soil compactness and improves the aeration in addition to the supply of essential plant nutrients and organic matter and increase soil microbial establishment along with accumulation of excess humus content (Giraddi, 1993). The population of bacteria in microbially enriched manures increased by 65 per cent as compared to the manures before enrichment. This was due to the carbon and nitrogen contents of manures and the moisture provided for the enrichment which also nourishes the microbes (Rajani *et al.,* 2001). Bio-fertilizers are widely accepted as low cost supplements to chemical fertilizers and no deleterious effect either on soil health or environment (Bhagyaraj and Suvarna, 1999). Consortium biofertilizers are defined as substances containing living microbes, which when applied to seed, plant, or soil promote growth by the supply of essential nutrients such as N, P and other mineral nutrients. Cucumber (*Cucumis sativus* L.) is an important salad vegetable crop which is cultivated throughout the country. Immature tender fruits of cucumber are universally useful in the culinary preparation of raitha, salad and pickle. There is a great demand for this crop throughout the year. Hence, an attempt was made to study the effect of organic manures and biofertilizers on nutrient uptake and post harvest soil nutrient status in cucumber var. Green long.

MATERIALS AND METHODS

A field experiment was conducted at Koneripalayam village in Perambalur district, during two seasons *viz.,* season I (February-May) and season II (July - October).The field area consists of red soil and had a soil pH of 7.1 and electrical conductivity of 1.01 dSm^{-1} . The experimental field was situated at 11°13' N latitude and 78°52' E longitude at an altitude of 98 M above mean sea level in Perambalur District of Tamil Nadu, India. The experiment was laid out in a randomized block design with three replications and fifteen treatments *viz.,* T1 – (Control - RDF), T2 –FYM + CBF, T3 –FYM + CBF + Se (5µg) as soil application, T4 –FYM + CBF + Se (10µg) as soil application, T5 – FYM + CBF + Se (20µg) as soil application, T6 –FYM + CBF + Se (5µg L^{-1}) as foliar application, T7 –FYM + CBF + Se (10µg L^{-1}) as foliar application, T8 –FYM + CBF + Se (20µg L^{-1}) as foliar application, T9 –EM + CBF, T10 –EM + CBF + Se (5µg) as soil application, T11 –EM + CBF + Se (10µg) as soil application, T12 –EM + CBF + Se (20µg) as soil application, T13 – EM + CBF + Se (5µg L^{-1}) as foliar application, T14 –EM + CBF + Se (10µg L^{-1}) as foliar application, T15 – EM + CBF + Se (20µg L^{-1}) as foliar application. The field was thoroughly ploughed and divided into plots of 2m x 2m. Three pits per plot were formed and the seeds were sown. The organic manures *viz.,* 25 t FYM ha^{-1}, 1 t EM ha^{-1} along with consortium of biofertilizers @ 2 kg ha^{-1} were incorporated at the time of last ploughing as per the treatment schedule. The selenium was applied in the form of sodium selenate through soil and foliar application as per treatment schedule in two split doses viz., 25 and 50 days after sowing. Thinning was done at ten days after sowing by retaining three seedlings per pit. The crop was irrigated every fifth day and proper drainage facilities were provided as the crop cannot withstand water logging. Necessary plant protection measures were carried out as per the recommendation. The fruits of cucumber were harvested when it was tender and green. The fruit takes 7 to 10 days from setting to reach the marketable size. The fruits were picked at every two days interval. The plant analysis was done from six plants, each plot were selected at random and pulled out at the time of harvest without damaging the roots washed with water to remove the dust particles. The plants were shade dried for one day and then dried in a hot air oven at 60°C. The dried plants were ground in a Willey mill having stainless steel sieves and used for analysis. The total nitrogen content was estimated by Microkjeldahl method suggested by Bremner (1960), the total phosphorus uptake by plant was estimated by using triple acid digestion method described by Jackson (1973) with photoelectric calorimeter and the total potassium content of plant was estimated using triple acid digestion method described by Jackson (1973) with a flame photometer. Then the nutrient content was calculated to kg/ha. The post harvest soil analysis was done by collecting the soil samples from each treatment plot after harvesting the crop. The

soil samples were air dried and analysed for available N (Subbiah and Asija, 1956), available P (Olsen *et al*., 1954) and available K (Jackson, 1973) in all the treatments and the results were expressed in kg ha^{-1}.The pooled mean data were statistically analyzed as per the standard procedure.

RESULTS AND DISCUSSION

The data on nutrient uptake by plants were found to be significantly increased which recorded nitrogen (148.87 kg ha^{-1} in first season and 161.27 kg ha^{-1} in second season), phosphorus (33.90 kg ha^{-1} in first season and 36.31 kg ha^{-1} in second season) and potassium uptake (156.21 kg ha^{-1} in first season and 169.14 kg ha^{-1} in second season) in the treatment T_{15} which received EM @ 1 t ha^{-1} + CBF @ 2 kg ha^{-1} + Se (20µg L^{-1}) as foliar application (Table 1). The uptake of plants was found to be minimum in the treatment which received FYM @ 25 t ha^{-1} + CBF @ 2 kg ha^{-1} (T_2). Application of enriched manure helped in enhancing nutrient levels in terms of primary and secondary nutrients and plays significant role in enhancing the soil fertility. Nitrogen uptake in plants is essential as it is the major constituent in the synthesis of proteins, enzymes, chlorophyll and nucleic acids. The increased nitrogen levels and uptake due to the application of organic manures was reported in the earlier findings of Joseph *et al*. (2007) in chickpea and Suresh *et al*. (2010) in tomato. Increased phosphorus uptake was also greatly influenced by the application of enriched manure along with the biofertilizers. Microorganisms such as, phosphobacteria present in the consortium biofertilizer plays a major role in the solubilisation of phosphorus and in addition helps in utilization of phytohormones such as, auxins, cytokinins, gibberellins and abscissic acid. These phytohormones when released into cropping systems promote better plant growth and possibly increase the P uptake. The reason for increased P uptake could also be due to decomposition of organic acids from the organic amendments applied. Similar findings were also reported by Laldinthar and Dkhar (2015).

The increased potassium uptake could be attributed due to the richness of enriched manure and consortium biofertilizers applied. These organic amendments with its humus forming and potash solubilizing microorganisms helps in improving the physico-chemical properties of the soil. The concentration of K in leaves could be increased due to the effect of solubilization of certain organic acids released by microorganisms in consortium biofertilizer. In the present study, plants supplemented with 20 µg $L^{-1}$of selenium recorded the maximum accumulation in fruits. This might be due to the foliar spray of selenium in the form of sodium selenate (Na_2SeO_4) which recorded to be efficient due to the accumulation of selenium through sulfate transport pathway coupled to a H + -ATPase as reported by Zhao *et al*. (2010).

Post harvest nutrient status of soil

The data on post harvest nutrient status of soil suggests that organic manure application favours better nutrient retention and utilization of N, P and K (Table 2). In the present investigation, nitrogen (174.61 kg ha^{-1} in first season and 181.20 kg ha^{-1} in second season), phosphorus (17.45 kg ha^{-1} in first season and 20.61 kg ha^{-1} in second season) and potassium (298.14kg ha^{-1} in first season and 319.05 kg ha^{-1} in second season) recorded was the highest in the treatment which received FYM @ 25 t ha^{-1} + CBF @ 2 kg ha^{-1} + selenium (20µg) as soil application. The least was found in the treatment that received enriched manure @ 1 t ha^{-1} + consortium biofertilizer @ 2 kg ha^{-1}. Among the various organic manures tested in two seasons, farmyard manure increased the nutrient availability due to efficient decomposition and mineralization of nutrients from source to sink. Dutta *et al*. (2016) reported that higher residual soil nutrient status might be attributed to increased microbial activities in the root zone which decomposes organic manures and also fixed unavailable form of mineral nutrients into available form in soil fulfilling the crop requirement. These findings are similar to those Moakala *et al*. (2015) in broccoli. The increased availability of N, P, K in soil could be influenced by the solubilizing potential of microorganisms such as, *Azospirillum*, phosphobacteria and potash solubilizing bacteria present in the consortium biofertilizers and also increased the solubility due to production of organic acids. The soil nutrient status after harvest was positively influenced by the bacterial population and soil physico-chemical properties. Increase in organic matter content due to the application of organic manures showed a positive correlation of soil organic carbon and soil phosphorus. These findings are in accordance with that of

Opheusden *et al*. (2012). Hence, it can be concluded that among all the three organic manures tested, application of EM @ 1 t ha^{-1} + CBF @ 2 kg ha^{-1} as soil application combined with Se (20µg L^{-1}) as foliar application was found to the best treatment in increasing the nutrient uptake and the treatment which received FYM @ 25 t ha $^{-1}$ + CBF @ 2 kg ha $^{-1}$ + Se (20µg) as soil application was proved to be superior in respect of post harvest soil nutrient status .

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Table 1.Effect of organic manures and selenium on nutrient uptake in cucumber var. Green Long

Tr. No	Treatment details	Nitrogen (kg ha^{-1})			Phosphorous (kg ha^{-1})			Potassium (kg ha^{-1})		
		Season I	Season II	Pooled Mean	Season I	Season II	Pooled Mean	Season I	Season II	Pooled Mean
T_1	Control (80:50:50 Kg NPK ha^{-1})	97.89	101.35	99.62	18.66	22.11	20.39	101.70	105.42	103.56
T_2	FYM + CBF	77.40	80.62	79.01	14.85	18.20	16.53	80.37	84.20	82.29
T_3	FYM + CBF + Se (5µg) as soil application	93.16	95.20	94.18	17.45	20.76	19.11	93.40	97.95	95.68
T_4	FYM + CBF + Se (10µg) as soil application	115.65	122.46	119.06	24.22	26.22	25.22	117.66	121.83	119.75

T_5	FYM + CBF + Se (20µg) as soil application	123.78	131.40	127.59	27.21	28.45	27.83	126.40	132.16	129.28
T_6	FYM + CBF + Se (5µg L^{-1}) as foliar application	130.42	139.85	135.14	28.76	31.14	29.95	136.10	144.32	140.21
T_7	FYM + CBF + Se (10µg L^{-1}) as foliar application	119.97	128.83	124.40	26.36	27.65	27.01	124.12	128.42	126.27
T_8	FYM + CBF + Se (20µg L^{-1}) as foliar application	138.24	149.06	143.65	31.16	33.18	32.17	144.21	156.27	150.24
T_9	EM + CBF	84.19	89.38	86.79	16.12	19.50	17.81	87.14	91.36	89.25
T_{10}	EM + CBF + Se (5µg) as soil application	110.39	119.18	114.79	23.85	25.18	24.52	115.33	118.40	116.87
T_{11}	EM + CBF + Se (10µg) as soil application	136.30	145.37	140.84	30.12	32.76	31.44	141.87	152.08	146.98
T_{12}	EM + CBF + Se (20µg) as soil application	144.10	153.43	148.77	32.45	35.04	33.75	150.36	162.60	156.48
T_{13}	EM + CBF + Se (5µg L^{-1}) as foliar application	102.11	112.80	107.46	20.12	23.85	21.99	108.10	111.87	109.99
T_{14}	EM + CBF + Se (10µg L^{-1}) as foliar application	128.54	136.70	132.62	28.45	30.28	29.37	133.74	139.53	136.64
T_{15}	EM + CBF + Se (20µg L^{-1}) as foliar application	148.87	161.27	155.07	33.90	36.31	35.11	156.21	169.14	162.68
S.ED		2.09	2.15	2.12	0.60	0.62	0.61	2.61	2.73	2.67
CD (p=0.05)		4.18	4.30	4.24	1.19	1.23	1.21	5.22	5.46	5.34

Table 2: Effect of organic manures and selenium on post-harvest available soil nutrients in cucumber var. Green Long

Tr. No	Treatment details	Nitrogen (kg ha^{-1})			Phosphorus (kg ha^{-1})			Potassium (kg ha^{-1})		
		Season I	Season II	Pooled Mean	Season I	Season II	Pooled Mean	Season I	Season II	Pooled Mean
T_1	Control (80:50:50 Kg NPK ha^{-1})	152.21	159.48	155.85	13.28	16.14	14.71	265.12	278.70	271.91
T_2	FYM + CBF	140.13	146.27	143.20	10.65	13.28	11.97	237.25	250.32	243.79
T_3	FYM + CBF + Se (5µg) as soil application	145.59	152.36	148.98	11.72	14.47	13.10	251.19	263.62	257.41
T_4	FYM + CBF + Se (10µg) as soil application	161.17	170.05	165.61	15.26	18.30	16.78	279.18	294.79	286.99

T_5	FYM + CBF + Se (20µg) as soil application	174.61	181.20	177.91	17.45	20.61	19.03	298.14	319.05	308.60
T_6	FYM + CBF + Se (5µg L^{-1}) as foliar application	149.96	157.04	153.50	12.64	15.58	14.11	259.75	274.12	266.94
T_7	FYM + CBF + Se (10µg L^{-1}) as foliar application	156.68	164.33	160.51	14.37	17.20	15.79	272.10	285.90	279.00
T_8	FYM + CBF + Se (20µg L^{-1}) as foliar application	170.82	178.53	174.68	16.93	20.04	18.49	292.96	314.10	303.53
T_9	EM + CBF	136.70	143.79	140.25	10.14	12.60	11.37	230.29	241.81	236.05
T_{10}	EM + CBF + Se (5µg) as soil application	142.96	148.82	145.89	11.19	13.85	12.52	246.40	259.11	252.76
T_{11}	EM + CBF + Se (10µg) as soil application	147.85	155.18	151.52	12.24	15.12	13.68	257.13	270.32	263.73
T_{12}	EM + CBF + Se (20µg) as soil application	154.88	162.84	158.86	13.92	16.70	15.31	269.77	283.48	276.63
T_{13}	EM + CBF + Se (5µg L^{-1}) as foliar application	159.41	167.93	163.67	14.90	17.84	16.37	276.84	292.27	284.56
T_{14}	EM + CBF + Se (10µg L^{-1}) as foliar application	164.90	172.53	168.72	15.79	18.86	17.33	283.87	301.18	292.53
T_{15}	EM + CBF + Se (20µg L^{-1}) as foliar application	168.13	175.40	171.77	16.37	19.48	17.93	288.40	307.62	298.01
S.ED		1.09	1.12	1.11	0.25	0.27	0.26	2.19	2.21	2.20
CD (p=0.05)		2.18	2.24	2.21	0.49	0.53	0.51	4.37	4.41	4.39

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RESEARCH ARTICLE

Evaluation of Immunostimulatory Potential of Rhamnolipid Biosurfactant from *Pseudomonas aeruginosa* in *Cirrhinus mrigala*

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ABSTRACT

Currently, the increasing consumer concern about the residues of antibiotics, hormones, growth promoters, and the risk of growth of antibiotic resistant strains has led to the use of immunostimulants in aquaculture. In this study, the immunostimulatory effect of normal and curd rhamnolipid biosurfactant from *Pseudomonas aeruginosa* was assessed with *Cirrhinus mrigala*. The immunostimlatory effects of rhamnolipid were determined from differences between treatment and control groups in survival rate, Heamoglobulin rate, total RBC, total WBC, and packed cell volume (PCV) values. A challenge test was conducted using *Cirrhinus mrigala* from each group (6 fishes) by intraperitoneal injection with 0.5 ml suspension culture of the pathogen *Vibrio parahaemolyticus* (10^8 bacteria ml^{-1}). The mortality rate was noted for 7 days post-challenge. Both normal and cured cells showed a significant increase in hematocrit values and leucotrit values. The survival rate was significantly increased in both, with challenge, when compared with control. It may be concluded that, biosurfactants can be used as a growth enhancer, immunostimulant and a disease control agent in fish. It is recommended as a means of improving the common carp aquaculture production under certain conditions.

Keywords: Biosurfactant, *Pseudomonas aeruginosa*, *Cirrhinus mrigala*, *Vibrio parahaemolyticus,* immunostimulatory.

INTRODUCTION

Disease is one of the major constrains to aquaculture and limiting factor for economic and socio-economic development in India and as in many other countries of the world [1,2,3]. Some diseases have caused serious damage, not only the livelihood of fish farmers, but also, to the future development of the industry. Many diseases affecting present day aquaculture is resultant of intensification of culture practices without the basic perception of intricate balance between host, pathogen and environment [4,5]. In aquatic systems, disease management is a difficult proposition due to the unique ecosystem, where the pathogen is always looking for an opportunity when the health status of the host is compromised [6]. Bacterial fish diseases are most common in aquaculture and are one of the most difficult health problems to deal with and have been studied extensively in aquaculture [7,8,9]. One of the main bacterial pathogens in India, *Vibrio parahaemolyticus*, is known to cause a range of diseases in fish such as ulcerative abscesses in internal organs, haemorrahic ulcers on skin, fins and body leading to heavy mortality in aquaculture farms [10]. Different synthetic chemicals and antibiotics have been used to prevent or treat fish diseases with a partial success. Though, the excess use of antibiotics results in the environmental vulnerability, food safety problems and development of drug-resistant pathogens [11]. The limitations of antibiotics, chemotherapeutics and vaccines suggest that aquaculture disease management should emphasize on harmless, preventative, and lasting methods.

Immunostimulants are critical in eliciting immune responses capable of providing complete protection against certain pathogens. In recent days, the use of immunostimulants was introduced as a prophylactic measure [12]. Since such uses have so far not shown any of the negative side effects that antibiotics and live vaccines may have on the fish and on the environment, they are an attractive alternative way of controlling bacterial infections (13, 12). At present, a variety of immunostimulants that include a very heterogenous group of substances like levamisole, lipopolysaccharide, glucans, peptidoglycan and muramyl dipeptide on the immune response have been investigated in different fish species [14]. But knowledge on the use of rhamnolipid biosurfactant as immunostimulants is limited [15,16,17,18]. Therefore, screening of novel immunostimulants from the secondary metabolites (biosurfactants) of microorganisms could be advantageous to strengthen fish immune system and to reduce the quantity of antibiotics required to control infectious diseases. Hence the present research focus on the evaluation of immunostimulatory property of extracellular secondary metabolite rhamnolipid biosurfactant isolated from *P. aeruginosa* to fish (*Cirrhinus mrigala).*

MATERIAL AND METHODS

Pseudomonas aeruginosa isolated from sludge sample and enriched by inoculating into sterile mineral salt medium (MSM) consisting: 0.1% KH_2PO_4, 0.1% K_2HPO_4, 0.02% $MgSO_4{\cdot}7H_2O$, 0.002% $CaCl_2{\cdot}2H_2O$, 0.005% $FeCl_3{\cdot}6H_2O$ and 0.2% $NaNO_3$ and 3% glycerol as the main carbon and energy source, with the pH of the medium adjusted to 6.8 [19]. The cultivation condition for preparation of the seed culture was 30°C, at 200 rpm with an incubation time of 18–24 h. After seed culture preparation, a 2% cell suspension of 0.7 optical density (OD) at 600 nm, which corresponded to approximately 10^7 colony forming units (CFU) mL^{-1}, was inoculated into 500 mL baffled flasks containing 100 mL MSM. The broth cultures were incubated on a 200 rpm orbital shaker for 120 h at 30°C.The crude biosurfactant was extracted from the MSM containing normal and cured cells by a combination of acid and solvent extraction methods were used. In brief, after 5 days of culturing the isolates in glycerol-MSM, the culture (100 mL) was centrifuged at 10,000 rpm for 30 min at 4°C to remove microbial cells. The presence of surface active compounds in the supernatant was then verified using the oil spreading method as previously reported by Ndlovu et al. [20]. Subsequently, the supernatants were acidified to a pH of approximately 2 using hydrochloric acid [21] and were stored overnight at 4°C in order to precipitate the biosurfactant compounds. The precipitate was then harvested by centrifugation at 10,000 rpm for 30 min at 4°C, and the pellet was washed with 50 mL of distilled water with the pH adjusted to 7.5 [21]. The respective insoluble fraction was then lyophilised and dissolved in 15% (*v*/*v*) methanol transferred into analytically weighed sterile vials and lyophilised again. The extracts were analytically weighed and

dissolved in 15% methanol to obtain a 1.00 mg mL^{-1} concentration, which was used for immunostimulatory activity. The role of plasmids analysis in the biosurfactants production was confirmed by curing the plasmid with acridine orange at a concentration of 500 µg /ml which was added to the culture broth and incubated for 12hrs [22]. The crude biosurfactants and plasmid cured cells were screened for immunostimulant studies.

The common carp, *Cirrhinus mrigala*. were obtained from a Private fish farm, Chennai, Tamil Nadu, India and acclimated to the laboratory conditions for 15 days in fish tank (4 X 3 X 3). During acclimatization period, fish were fed ad libitum with rice bran and ground oil cake in the form of dough once daily. Water was replaced every 24 hours after feeding in order to maintain a healthy environment for the fish during both acclimatization and experimental period. This ensures sufficient oxygen supply for the fish and the environment is devoid of any accumulated metabolic waste. After acclimatization, fish with an average length 8.5 cm and average weight of 7.0 g were selected for the study. Fish were divided into 6 groups (ten fishes/group). Fish diet it contained 30% crude protein, 3.7 Kcal/g of metabolizable energy, 3.4% fiber and 7.03% fat as well as vitamins and minerals in the form of dry pellets. Two different concentration (50 and 100 ppm of crude BS and Cured BS) containing biosurfctant were used and mixed thoroughly with the prepared basal fish diet during its preparation, one control is maintained [23]. Survival rate was recorded during the course of the feeding experiment for all treatment replicates. The Heamoglobulin rate, Total RBC, Total WBC and packed cell volume were carried out according to the method of Stoskoph [24]. These parameters were determined from blood samples, collected after the first and second phases from the caudal vein of 20 fish from each treatment group (5 from each replicate) using sterile syringes with saturated EDTA.

Challenge of infections was carried out three times on the treatment groups: after feeding on the test diets for one month and at the end of the experiment. Six fishes from each treatment group and from the control (5 from each replicate), were clinically examined and blood samples bacteriologically tested and determined to be free from bacterial infection, were then artificially infected by intraperitoneal injection with 0.5 ml of culture suspension of pathogenic *Vibrio parahaemolyticus* containing 10^8 bacteria ml^{-1} that were previously isolated from moribund fish and studied for pathogenicity. The estimation the level of total Hb, Total RBC, Total WBC and PCV were carried out according to the method of Stoskoph [24]. A culture suspension of *Vibrio parahaemolyticus* was prepared by culturing in agar for 24 h, washed and suspended in saline (0.85%) and counted using MacFirland standard tubes (No.1). The relative level of protection (RLP), among the challenged fish was determined according to Ruangroupan *et al*., [25] using the following equation; RLP = 100 - % immunized mortality ÷ % control mortality x 100.

RESULT AND DISCUSSION

Pseudomonas sp, is an outstanding and natural crude oil degrader, reported in the literature, which is widespread in nature and can degrade wide range of xenobiotics [26,27,28]. Eniola *et al*., [29] observed that *Pseudomonas aeruginosa* had higher degradation potential among microbes collected from soil contaminated by used engine oil in Nigeria. The different particle of bacterial source, such as lipopolysaccharides, lipoproteins and glycoproteins, as well as by enzymes produced by immune cells, such as cytokines, transferrin, lysozyme and interleukins [30,31] are involved in the activation of native immune system in fish. Microbial secondary metabolites are the defensive subcellular component has received more attention in management of fish disease. Previously, Jeyanthi amd Revathy, [17] noticed that the metabolites of rhamnolipids of *Pseudomonas putida* were successful for the stimulation of immunity and the prevention of *Aeromonas hydrophila* infections *Labeo rohita* fishes. The present study demonstrated that the rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* strain was able to influence haematological components and challenges of infection in *Cirrhinus mrigala*. The Hb, RBC, WBC and PCV level were 7.06 %, 0.38 10^6/cu.mm, 20.8 10^3/cu.mm, 30% estimated in control rats, respectively. At end the experimental period the *V. parahaemolyticus* treated fishes showed the drastically changes of these parameters from control animals 4.89%, 0.24 10^6/cu.mm, 31.3 10^3/cu.mm, 39.2%, respectively. In the present study, both the concentrations of crude and curd biosurfactant in *V. parahaemolyticus* administarted fishes, the level of Hb, total RBC, WBC and PCV were improved

(Fig 1-4). These could be attributed to the fact that, the biosurfactant used increased the blood parameter values as a result of hemopiotic stimulation. These results supported the results of Jeyanthi amd Revathy, [17] and Ndlovu et al., [20]. Several investigations have shown the immumomodulatory or immunostimulatory activity from biosurfactants in fishes. This result is supported by another study [32,33], which found that there was an increase in the WBC count when *Labeo rohita* juveniles were treated with immunostimulants like levamisole and ascorbic acid. The increase in total white blood cells, neutrophils, lymphocytes and monocytes following feeding of algal and herbal diets supports the notion of antimicrobial properties of the algae *Euglena viridis* [34] and traditional herbal medicines [33]. The fish blood packed cell volume is an indicator of the health status and can be helpful in detecting any abnormal changes including improvement through the use of immunostimulants. Anemic fish may have hematocrit values as low as 10%. The reduced hematocrit values may indicate that the fish are not eating properly or were suffering from infections [35]. The biosurfactants treated group in the current work, exhibited significantly higher survival throughout the experimental period and higher mortality rate post has registered in pathogen alone treated group when compared with the control group (Fig 5). Earlier studies also revealed a peptide FK-565 (heptanoyl-g-D-glutamyl- (L)-meso-diaminopimelyl-(D)-alanine) isolated from the culture supernatant of *Streptomyces olivaceogriseus* into rainbow trout (*Salmo gairdneri*) increased their resistance to *Aeromonas salmonicida*, following the activation of phagocytic cells [36]. It may be concluded that, rhamnolipid biosurfactant acts as an immunostimulant and a disease control agent in fish. It may be recommended as a dietary supplement in order to improve aquaculture production, after further studies are running to evaluate cost-benefits.

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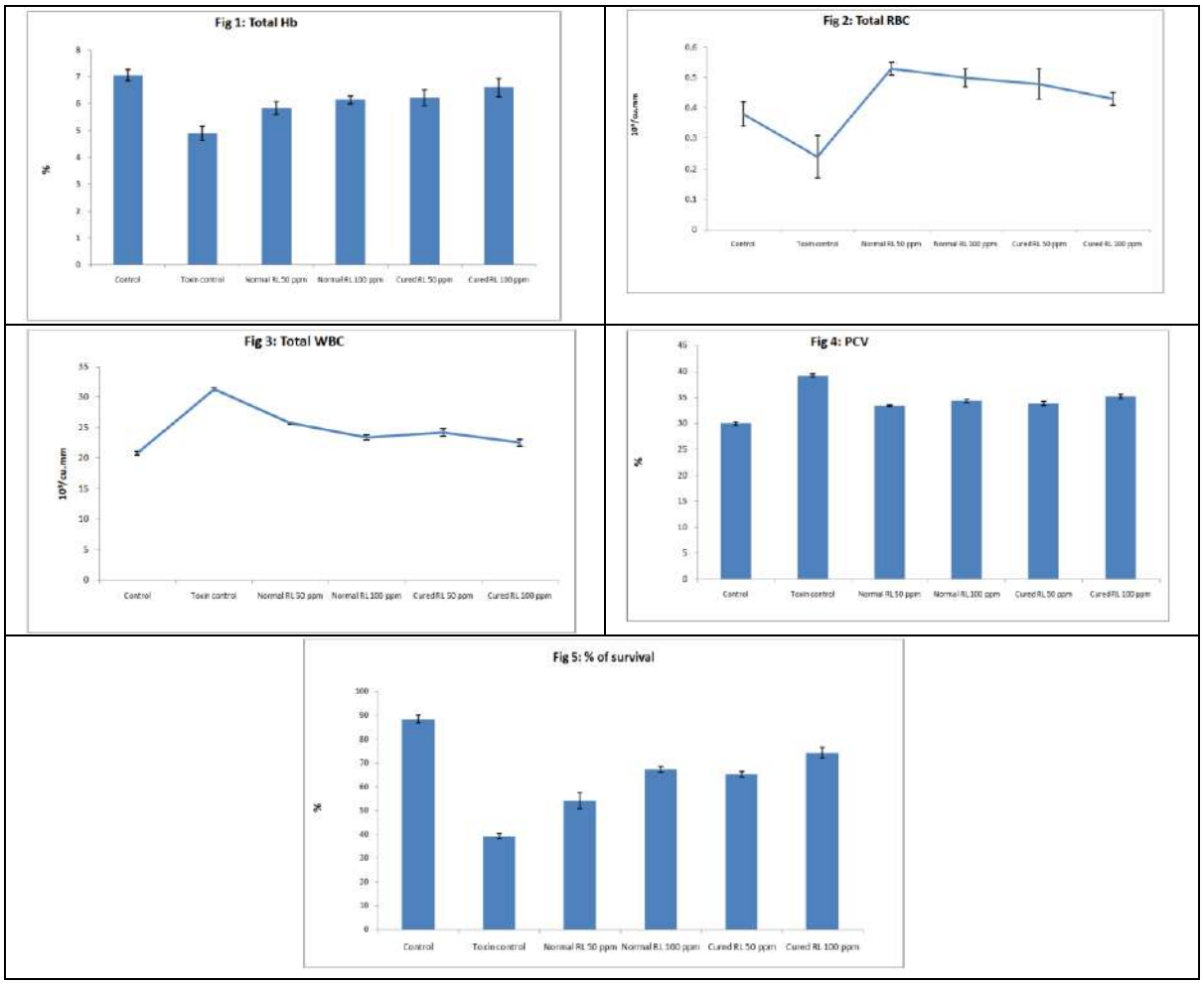

RESEARCH ARTICLE

Metabolomics – A Tailored Approach in Periodontics

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ABSTRACT

Periodontal disease progression and treatment is based on the factors ranging from the macroscopic to even the microscopic scale. The body's own metabolome (collection of tiny molecules and metabolic products of cells and tissues) isamongst one of the many etiological agents contributing to periodontal disease. Being regulatory in nature, the association of these molecules has a substantial impact on the genesis and progression of disease, which differs in each individual based on their phenotype. Exploration of this relationship and implementing it to the field of periodontitis diagnostics and risk prediction can result in a tailored level of care for patients in the future.

Keywords: Periodontitis, Pathogenesis, Individualized medicine, metabolomics, periodontal medicine.

INTRODUCTION

Metabolomics, a new platform for biomarker discovery, tries to capture smaller biological molecules such as simple amino acids, lipids, carbohydrates, nucleotides, and other transitional metabolites systemically. Because proteins and metabolites are synthesized in accordance with genetic diversity and transcriptional changes, they can give immediate "snapshots" of a cell's or organism's status. They can alter swiftly in response to external stresses like exercise or directly by ingesting meals or other chemicals. An increasing corpus of research reveals that microscopic proteins and metabolites could play an unanticipated role in the regulation of physiological activities such as blood

pressure and energy homeostasis. Although less evolved than other techniques, it can provide insight into the full complexity of a specific disease as well as aid in the identification of new biomarkers. Exploring this link and applying it to periodontitis diagnostics and risk prediction may not only give information on biology but also highlight potential therapeutic breakthroughs suited to specific phenotypes and genotypes [1].

THE, METABOLITES, METABOLOME AND METABOLOMICS

Metabolites are compounds that help organisms exchange messages and convey signals; their composition fluctuates depending on genetic make-up and ecological stressors. They are the end result of activities that occur within a cell. As a consequence, the metabolome includes all metabolites detected in a biological cell, tissue, or organism. Since metabolites are essential in cellular life and function, metabolomics research could provide insight on an organism's biological functions, including its genetic blueprint and current environmental exposures [2]. The biology and the respective methodologies of these systems may be investigated at several levels, beginning with the most fundamental, the genome, and ending with the most functional, the metabolome. This developing subject is obviously connected to genomes, transcriptomics, and proteomics[Figure 1]. Furthermore, metabolomics gives a unique and direct view of the functional consequence of an organism's activities that are essential for it to survive, grow, and respond to internal and external stimuli or stress, such as diseases and medications. Metabolomics plays an integral role because, unlike other "omics" parameters, concentration levels of different metabolites tend to epitomize a core biochemical activity along with the cells / tissues homeostasis. In a nutshell, metabolomics accurately portrays the molecular phenotype [3].

HISTORY

Metabolomics, along with genomes and proteomics, is the youngest member of the systems biology trio. The word "metabolome" was first used in 1998, and metabolomics was still considered an emerging subject in 2010. Roger Williams' demonstration in the late 1940s showed that each individual could have a "metabolic profile" reflected in their body fluids. Several decades later, technological breakthroughs permitted quantitative measurements of metabolites. Horning et al. created the phrase "metabolic profile" in 1971 when they demonstrated that chemicals in urine or tissue extracts could be quantified using gas chromatography-mass spectrometry (GC-MS)[4]. Simultaneously, nuclear magnetic resonance (NMR) technology was being utilized to identify metabolites in raw biological samples. As greater magnetic fields and magic angle spins were used, the sensitivity of this technology grew with time[5]. Professor Jeremy Nicholson highlighted the potential application of NMR spectroscopy in the diagnosis of diabetes in 1984 [4].

WHY THE SEARCH FOR NEWER METHODS?

Periodontal disease diagnosis and treatment is subjected to a number of risk factors such as smoking. The traditional diagnostic methods often lead to delayed diagnosis, further delaying the treatment aspect and leading to tooth loss. The dearth of an all-inclusive and accurate diagnosis has led to the “omic” based diagnostic methods favoring a customized treatment plan as per requirement [3]. The sequencing of various species has advanced significantly, as have the tools used to evaluate cell products such as proteins and metabolites. The study about the metabolome helps to draw direct inferences on how the body behaves in health and disease, thus giving substantial evidence to support the hypothesis that metabolomics can help device better treatment strategies for periodontal diseases [6]. This comparatively novel method of evaluating the body’s essential macro and micro metabolites and their associated intracellular reactions helps to evaluate the deviation from the normal for any of these metabolites leading to an underlying disease, including but not limited to the periodontium [7].

THE RELATION – PERIODONTITIS AND METABOLOMICS

Recent microbiome researches have indicated that multiple pathogens are involved in the dysbiosis caused as a result of periodontitis. The host's ability to respond and defend is entirely dependent on how well the defense system of the body comes into play. The field of diagnostic medicine is related to mostly traditional methods of disease diagnosis namely radiographic interpretation, microbiological testing as well as clinical diagnosis, all having their own advantages as well as disadvantages. With the advent of a newer diagnostic and ‘cut to fit’ treatment era, it

is important to make some amends in the existing treatment strategy [8]. According to Bartold et al in 2018, the cornerstone of almost any treatment model is its ability to develop and administer a tailored treatment approach. To bring this notion to actuality advanced diagnostic procedures that take into account all or most of these elements are crucial, and metabolomics analysis offers tremendous potential in this context [9]. The shift in the oral microbiome occurs not immediately but rather follows and takes a considerable amount of time. This gradual shift of microbial population from a symbiotic one to a pathological one leads to the destruction caused by periodontitis. According to the most recent model of dysbiosis provided by Van Dyke et al., the continuous degradation does not just entail changes in the microbial population but also gets input from changes in the individual's metabolomics. Few of the most recent literature supports the notion that metabolomics can indeed favor how the conventional treatment strategies can be altered [10]. In 2017, Sakanaka et al. published a research that evoked a distinct metabolomic footprint that could conclusively be employed to predict or diagnose periodontal disease activity. Cadaverine, hydrocinnamate, and arginine, proline, and lysine were among the metabolites studied. Van Dyke et al. used machine learning in the year 2020 to assess, stratify, and interpret the multitude of information gathered from metabolomic research in distinct phenotypes. Depending on the outcomes, schematics for diagnosis and interventional strategies could be devised. Also, an evaluation of the prognosis and risk of periodontal disease in an individual may very well be constructed [11]. Systemic illnesses such as diabetes, CVS diseases, autoimmune diseases, and periodontitis have a bidirectional link. The onset of illness varies depending on the individual's metabolic profile, therefore, making it a daunting task to tailor the existing therapeutic modalities. To address this problem, therapeutic approaches must be established in such a manner that they cater to every facet of a particular disease profile. This also necessitates the requirement for a "personalized" treatment approach to be instilled in periodontics [12].

FORERUNNERS IN METABOLOMICS

The oral cavity has at least five communities: teeth; saliva; the dorso-lateral aspect of tongue; gingival sulcus and periodontal pocket; and the remaining epithelial surfaces of the oral mucosae. All of these harbor different micro-organisms that cause periodontitis once dysbiosis occurs. The metabolites generated from these microbes determine the amount of destruction being caused in the periodontium. Saliva amongst them is a reliable source for the study of metabolites produced. Approaches such as liquid or gas chromatography/mass spectrometry (MS) enable a critical appraisal of the metabolite values, though these mentioned mechanisms have quite a low threshold in the detection of particular metobolites [13]. These approaches do not permit for such study of untargeted molecules. This disadvantage is mitigated by using an untargeted metabolomics technique that incorporates proton nuclear magnetic resonance spectroscopy (NMR). The advantage of employing NMR spectroscopy to analyze pure saliva is that no pre-treatment is essential.On the other hand, MS-based approaches not needing post-extraction parting of the metabolites identified have acquired widespread approval and application in salivary metabolomic investigations [14]. Exploration into the metabolomics changes caused as a result of diseases, can lead to identification of various biomarkers and disease specific pathogenesis. Multiple contributing factors, such as clinical characteristics, age, sex, and comorbidities, give a promising path for further study. [12]Various methods exist to do so namely handheld devices, metered devices, and even artificial intelligence has been put into use to do so [15] .The study and detection of existing or modified metabolites produced by periodontal pathogens or the hosts, using microfluidics, is indeed a promising avenue for research in the field of metabolomics. Adhering to a strict and properly chosen protocol for the study of metabolites is a must post the selection of armamentarium, methodical workflow, and the required technique. This prevents any inadvertent experimental mistakes while also assuring a seamless conclusion of each cycle. These stages might be described as a systematic and scientific process that begins with a patient reporting clinical symptoms and concludes with ailment diagnosis and care [Figure 2].[12,16].

THE SILVER LINING

Metabolomics is indeed a silver lining when it comes to devising newer treatment modalities. But this new method does come with its share of drawbacks too.

Understanding the concept:

Despite the amount of research done on metabolomics, a decade later it is still considered as an emerging field only. Other 'omics' based technologies have gathered quite an interest and have a number of works published and still underway on it. A thorough analysis of all the data obtained need to be gathered and this data needs to be simplified in order to be put to use [13,17].

Identification of Metabolite

Even as more spectrum information becomes available and recorded in the literature and spectral databases, identifying many metabolites remains challenging, particularly when NMR spectrometry is used. Despite the presence of several open-access and commercial databases, there is currently no software accessible to assist in the identification process. It's the first issue that needs addressing when adopting the metabolomics approach [5, 18].

Statistical Analysis Improvement

In the future, relevant analysis tool handling must be addressed, as must validation of innovative approaches. The present statistical methodologies used in many metabolomics projects are clearly inadequate. In having to conduct a detailed examination, data from several platforms is essential to analyze the individual's compound biochemical profile. Professional statisticians who can run experiments and effectively handle the vast amount of data gathered throughout the process are in high demand [18].

Protocol standardization

Another hurdle is regulating the condition within which biological samples are collected, manipulated, and assessed. Many biological samples degrade and become unstable during storage. As a result, their elemental composition might vary massively. Inconsistencies in the protocols used for sample handling and evaluating the spectral data obtained may result in poor reliability and/or inaccurate analysis of the data available [17].

Costing issues

When cost is accounted, the operational prosperity of metabolomics methodologies is relatively high, making it a daunting task. A conclusive study entails data analysis from various methods, and the volume of data accessible after experimental analysis is substantial. As a consequence, if an investigator needs to expand a laboratory charge of implementing such an experiment, they must analyze the economic feasibility as well [12, 13].

CONCLUSIONS AND PERSPECTIVES

Metabolomics is unquestionably a potent and effective technique for a clearer grasp of biological systems, disease etiologies, and the impacts of environment, lifestyle, and food on physiological status, biological targets, and pharmaceutical effectiveness and toxicity. The concept certainly can support the identification of pathologies and/or toxicity biomarkers, which may contribute to enhanced characterization and stratification of both patients and diseases due to their direct correlation with phenotype and affiliations with genotype. Metabolomics might potentially be a helpful and strong technique for assessing novel treatment targets and novel pathways. Metabolomics is poised to become a significant role in biomedical sciences and drug development for all of these reasons.It might also be a difficult route to personalized medicine and therapy, which is anticipated to be the paradigm of medicine and patient care. Metabolomics is a basic instrument with immense potential for contributing as a rich source of presumptive information, but these merits are clearly reliant on how the tool is implemented and how the results were analyzed. Various characteristics, such as phenotype, nutrition, microbiota, age, and ethnicity, could have a significant effect on metabolomics data, contributing to misconstrued results. This characteristic, on the other hand, may be used to judge the quality and reproducibility of clinical and preclinical simulations. Metabolomics' future state would most likely be in its incorporation with other new, well-defined, and/or older strategies adopted in diagnostics or drug discovery. Nonetheless, metabolomics has the capability to significantly improve existing standards by exploring and integrating critical data on metabolism, phenotype, and lifestyle. Being

used in integrated multidisciplinary platforms for diagnostics and individualized medicine is a near certainty which will be well established in the coming years. Although this approach has reached maturity and can be used routinely in research, several improvements remain to be done, including the advancement of instruments with relatively high selectivity and sensitivity, the combination of numerous technological approaches, the development of strong chemometric tools, and the assimilation of metabolomics with other "omics" sciences for the comprehensive and integrated interpretation of disease-specific pathways.

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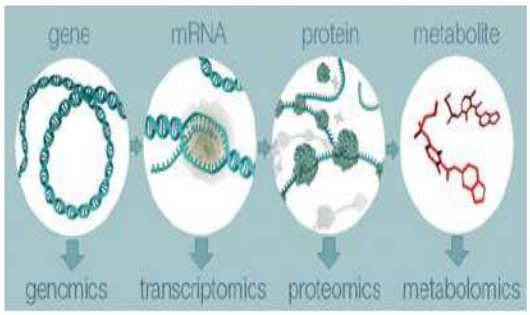

Figure 1: Overview from genomics to metabolomics

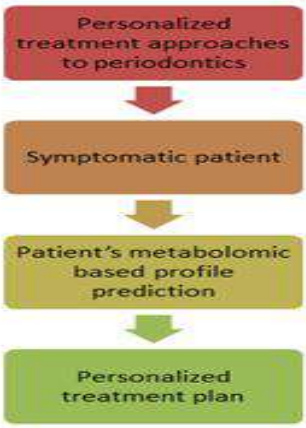

Figure 2: From processing to outcomes, a systematic method to metabolomics analysis

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RESEARCH ARTICLE

Prescription Pattern in Inpatients of Cardiovascular Disease at Tertiary Care Hospital in Dharmapuri District: Retrospective Observational Study

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ABSTRACT

To study the Prescription Pattern in Inpatients of Cardiovascular Disease at Tertiary Care Hospital in Dharmapuri District: Retrospective Observational Study. The study was a retrospective observational study conducted in a tertiary hospital, Dharmapuri. This study duration was six months January to July 2022. The study analyzed and documented for patients demographic details, disease prevalence, indication, co-morbidities and prescribing pattern of the physician, A total of 102 patients were analyzed. In our study most of the patients diagnosed with CVD's were of the age group of 40-49 (45.1%) years. 71.6 % male had a high frequency of cardiovascular disease incidence as a compared to 28.4% of female patients. Our finding indicated that 18.6% patients were reported with hypertension and 16.6 % patients were diagnosed with Coronary Artery Disease were most frequently diagnosed disease. The use of calcium channel blockers, beta blockers, diuretics, angiotensin-converting enzymes was very common, diabetic mellitus, asthma and anaemia were the commodities associated with cardiovascular diseases.
the present study appear that most of drugs were prescribed rationally according to the recent treatment guidelines except the under use of angiotensin-converting enzymes and ARB's in DM and hypertensive patients. Standard medical treatment guidelines should be spread among practicing physicians to motivate rational prescription.

Keywords: Prescription pattern, Coronary Artery Disease, Cardiovascular Disease

INTRODUCTION

Cardiovascular disease is a major health issue throughout the global and common origin of morbidity and mortality. According to WHO, CVD's are the number one source of death in worldwide. As estimated 17.5 million people were died from CVD's in 2008, 30% cases of all global deaths (WHO, 2009). Over 80% of cases deaths take place every year in middle and low-income countries like Bangladesh (BBS, 2009)[1], By the end of 2020, India is predicted to be heart related disease capital of the global with estimated rise of 111% of CVD's deaths. CVD's mortality rates in India are the global average (272 vs.235 per 1, 00,000 population). The elevated levels of blood cholesterol, excessive consumption of alcohol, hypertension, smoking, obesity and malnutrition etc. are some of the highly risk factors for cardiovascular disease.[2,3] The possible choice of treatment for the administration of cardiovascular drugs are ACE inhibitors, lipid lowering drugs, vasodilators, diuretics, calcium channel blockers and beta blocker etc.[4]population-based trends in medication use have important implications for patient health probed the trends in the drug use, however few of the studies have pondered the appropriateness of trends (shaila *et, al,* 2007). In this study, we determined the various co-morbidities amount the inpatient admitted to the cardiology department in tertiary care hospital in Dharmapuri district, tamilnadu.

MATERIALS AND METHODS

The study was a retrospective observational study conducted in a tertiary hospital, Dharmapuri. This study duration was six months January to July 2022. A total 102 patients who admitted under department of medicine and intensive unit with cardiovascular disease were included in the present study. Source of data was collected from patient's case history obtained from MRD. Patient's details such as patient name, sex, age, complaints, diagnosis and treatment were collected. The study was approved by the institutional ethical review committee. The database was computerized using MS Excel and descriptive results were expressed as counts and percentage.

RESULT AND DISCUSSION

Patient demographic

Out of 102 patients with cardiovascular disease were collected in this present study. 71.6 % male had a high frequency of cardiovascular disease incidence as a compared to 28.4% of female patients. In our study most of the patients diagnosed with CVD's were of the age group of 40-49 (45.1%) years followed by above 70 years (30.4) and 40-49 years (24.5%) age group.(Table 1)

Co-Morbid Assessments

Table 2 shows the various co-morbid assessments like Diabetic Mellitus, Hypertension, Renal disorders, Anaemia, Asthma/COPD, Others and none were seen among these patients. In total 102 patients 31 patients with (30.4%), 19 patients with hypertension (17.6%) and 19 patients with renal disorders (18.6%) most common co-morbid found in most of patients which high risk of cardiovascular disease morbidity and mortality.

Pattern of medical condition among the cardiovascular disease

Table 3 shows the different types of cardiovascular diseases diagnosed. 18.6% patients were reported with hypertension and 16.6 % patients were diagnosed with Coronary Artery Disease. Followed by other conditions were Myocardial infraction (12.74%), Ischemic heart disease (13.72%), Cardiac Myopathy (10.78%), Hyperlipidaemia (8.82%), Heart Failure (5.88), Stroke (3.92), Angina pectoris (8.82),

Drugs prescribed Pattern of different condition among the cardiovascular disease

Table 4 shows the prescription pattern of various category of drugs for treatment of cardiovascular diseases patient's namely antihypertensive drugs, lipid lowering agents, antiplatelet drugs, anti-anginals and diuretics. The usages of

these drugs were analysis and recorded. In this study most commonly used antihypertensive drugs were beta-blockers, calcium channel blockers and diuretics are given to patients with hypertension condition. Most of the physician's prescribe single product rather than combination drugs. Carvedilol (29.4%), amlodipine (80.3%) were the mostly preferred options for the patients. (Table 5) In the present study, rosuvastatin (16.6%) was given to most of the patients whereas the second option was atorvastatin (6.8%).(Table 5) In this study, (28, 4%) patients were only used clopidogrel, Aspirin and clopidogrel combination of drugs given to (14.7%) and aspirin (8.8%) and warfarin (18.6%) were prescribed. (Table 5) In this study, direct vasodilators were used common treatment for cardiovascular patients, isosorbide di nitrate (36.2%) was the commonly prescribed antianginal drugs, followed by ivabradine (25.4%) and nitro-glycerine (20.5%) was prescribed in patients. .(Table 5)In this study identify that most commonly prescribed diuretic drug was furosemide (51.9%), followed by the Hydrochlorothiazide (30.3%) and Mannitol (3.9%) was to the minority of the patients. .(Table 5)The present study found that non cardiovascular drugs prescribed to treat associated medical conditions were antiulcer drugs, antidiabetic drugs, bronchodilators and multivitamins as per the patients need. .(Table 5)

CONCLUSION

The current study appear that most of drugs were prescribed rationally according to the recent treatment guidelines except the under use of angiotensin-converting enzymes and ARB's in DM and hypertensive patients. The uses of ant-platelet and anticoagulated is use addition in the essential treatment as well as prevention of IHDs. Standard medical treatment guidelines should be spread among practicing physicians to motivate rational prescription,

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Table:1Patient demographic

Characteristic	No.(%) of Patients`
Sex	
Male	71.6
Female	28.4
Age	
20-39	24.5
40-49	45.1
Above 70	30.4

Table 2 Co-Morbid Assessments

Medical Condition	Number of patients	Percentage (%)
Diabetic Mellitus	31	30.4
Hypertension	18	17.6
Renal disorders	19	18.6
Anaemia	9	8.8
Asthma/COPD	18	17.6
Others	4	3.9
None	3	2.9

Table 3 Pattern of medical condition among the cardiovascular disease

Medical Condition	Number of patients	Percentage (%)
Hypertension	19	18.6%
Coronary Artery Disease	17	16.6%
Cardiac Myopathy	11	10.78%
Hyperlipidaemia	9	8.82%
Heart Failure	6	5.88%
Stroke	4	3.92%
Angina pectoris	9	8.82%
Myocardial infraction	13	12.74%
Ischemic heart disease	14	13.72%

Table 4 Drugs prescribed Pattern of different condition among the cardiovascular disease

Drug Therapy	Number of patients (n=102)	Percentage (%)
Beta blockers	64	62.75
Lipid Lowering Drug	26	25.49
Anti-Platelets	53	51.96
Anti anginal drugs	104	79.41
Calcium channel blockers	90	88.24
Diuretics	88	86.27
Miscellaneous	92	90.20

Table 5 different Category of drugs prescribed to the cardiovascular disease

Category of drugs	Treatment	Number of patients use antihypertensive drug	Percentage (%)
Beta blockers	Metoprolol	16	15.6%
	Nefedipine	18	17.6%
	Carvedilol	30	29.4%
	Metoprolol + Nefedipine	4	3.9%
Lipid Lowering Drug	Atorvastatin	7	6.8%
	Rosuvastatin	17	16.6%
	Fenofibrate	2	1.9%
Anti-Platelets	Clopidogrel	29	28.4%
	Aspirin	9	8.8%
	Aspirin + Clopidogrel	15	14.7%
	Warfarin	19	18.6%
Anti anginal drugs	Isosorbidinitrate	37	36.2%
	Nitroglycerin	21	20.5%
	Ivabradine	26	25.4%
Calcium channel blockers	Amlodipine	82	80.3%
	Diltiazem	5	4.9%
	Verapamil	3	2.9%
Diuretics	Furosemide	53	51.9%
	Mannitol	4	3.9%
	Hydrochlorothiazide	31	30.3%

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RESEARCH ARTICLE

Effect of Organic and Inorganic Liquid Fertilizers on the Growth and Yield of Sesamum (*Sesamum indicum* L.)

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ABSTRACT

A field experiment was carried out in the Experimental Farm of Department of Agronomy, Annamalai University, Annamalai Nagar, India during March-June 2021, to study the effect of foliar application of different organic and inorganic nutrients on the growth and yield of sesamum. This experiment was carried out in the randomized block design with three replications and comprised of ten treatments *viz*. Water, Panchagavya spray, Sea weed extract, Vermiwash, Humic acid, Fish meal extract, Bokashi spray, Banana pseudostem extract, DAP+NAA, Polyfeed. The experimental results revealed that foliar application of vermiwash @ 10 % increased the plant height (51cm), number of branches/plant (4.4), number of leaves/plant (66), LAI (1.3), yield (131 Kg/ha) and BCR (0.27) compared to water sprayed control.

Keywords: Bokashi, Banana pseudostem extract, Vermiwash, Panchagavya, LAI, Yield.

INTRODUCTION

Sesamum is commonly known as gingelly or til and popularly known as "Queen of Oilseeds and it is one of the ancient and third important oilseed crops of India next to groundnut and rapeseed/mustard. It is a versatile crop grown in varied environments from semiarid tropics, subtropics and in temperate regions. It is grown on residual soil moisture with low inputs, and is a good crop for rotations with an extensive tap root system. Globally, India

ranks first in the world and it is the largest producer of sesamum catering to the world market. In the year 2018-19, the production was 8.66 lakh tons from 19.47 lakh hectares with average productivity of 413 kg/ha. Sesamum was cultivated in 0.416 lakh hectares with production of 0.230 lakh tonnes with productivity of 555 kg/ha in Tamil Nadu during 2017-18. Sesamum seeds possesses lipid contents 48g, carbohydrates 25.7g, proteins 17g, fiber 14g and ash 6g approximately in 100g. Sesamum seed contains 44-57% oil, 18-25% protein and 13-14% carbohydrates. Sesamum oil possesses nutritional and medicinal values. Calcium, Phosphorus, Magnesium and Potassium minerals are present at larger amounts in sesamum oil. Besides it also has vitamins *viz*. Niacin, Thiamin, Riboflavin and vitamin B-6 (USDA nutrient database, 2015) [1]. Sesamum seeds contain two unique substances namely sesamin and sesamolin. These two substances having cholesterol lowering effect and able to prevent high blood pressure in humans. Sesamum oil is also known to maintain high density lipoprotein cholesterol (HDL) and lower low density lipoprotein cholesterol (LDL). Refined sesamum oil has antioxidant components like lignins allowing for greater shelf-life of foods plus improving their flavour and taste. Unsaturated fatty acids are rich in sesamum oil. Sesamum oil has 14% saturated fatty acids, 39% mono-unsaturated fatty acids and 46% poly-unsaturated fatty acids and omega 6 fatty acids are also rich in sesamum oil. The average yield of sesamum in India is low as compared with othercountries in the world.Sesamum yield gets reduce due to cultivation of sesamum in marginal and sub marginal lands, improper agronomic practices such as weeding, fertilizer application and physiological problems etc. The main hindrance in increasing the productivity is encountered with some physiological problems like flower drop and poor seed filling which seems to be associated with nutrient deficiency, hormonal imbalance etc. A well-managed crop can yield 1200 to 1500 kg/ha under irrigated and 800 to 1000 kg/ha under rainfed conditions (ICAR, 2013) [(2)]. Foliar application is credited with the advantage of quick and efficient utilization of nutrients, elimination of losses through leaching and fixation and regulating the uptake of nutrient by plants. Foliar application provides water to crops along with nutrients. Bokashi is a liquid product obtained after fermentation of fruit wastes with the help of EM and jaggery. Banana pseudo stem extract contain nutrients and can be used as liquid fertilizer. Foliar nutrition reduces the amount of fertilizer thereby reducing the cost of cultivation and also economizing crop production. Flower dropping is common in sesamum. In order to reduce it, to supply nutrients and to provide growth promoting substances various readily and newly available water soluble inorganic and organic liquid fertilizers were applied as foliar spray to study its effect on growth and yield of sesamum.

MATERIALS AND METHODS

A field experiment was carried out in the Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar, India during March – June 2021. The experimental field soil is clay loam in texture with a pH of 7.6. The experimental field soil comprised of low in organic carbon (0.40%), low in available nitrogen (211.5 kg/ha), medium in available phosphorus (20.2 kg/ha) and high in available potassium (308 kg/ha). The sesamum variety TMV 7 were sown in furrow lines with a seed rate of 5 kg/ha on 17th March, 2021. The spacing between rows were maintained with 30 cm. Two thinning operations were carried out in sesamum. The seedlings were thinned out at a spacing of 30 cm in between the plants at 30. This experiment was carried out in the randomized block design with three replications. The plot size was 4x5 m. This experiment comprised of ten treatments *viz*. Water spray (T_1), Panchagavya spray @ 3% (T_2), Biovita (Sea weed extract) spray @ 1% (T_3), Vermiwash spray @ 10% (T_4), Huminol gold plus (Humic acid) spray @ 1% (T_5), Fish meal extract spray @ 1% (T_6), Bokashi spray @ 5% (T_7), Banana pseudostem extract spray @ 2% (T_8), DAP @ 2% + NAA @ 40 ppm spray (T_9), Polyfeed (NPK 19:19:19) spray @ 1% (T_{10}). The Recommended rate of fertilizer (NPK 35:23:23 kg/ha) was applied through urea, single super phosphate and muriate of potash as basal dose for all the treatments. The various organic and inorganic fertilizer solutions were prepared at the required concentrations and applied on 30 and 45 DAS to the respective plots as per the treatment schedule by using hand operated knapsack sprayer with spray fluid volume of 500 lit/ha. The crop was harvested on 30th May, 2021 (80 days).

RESULTS AND DISCUSSION

Plant height

The plant height was significantly influenced by the treatments tested. Various treatments increased the plant height from 8 to 51 cm over water sprayed control. T_4- Foliar application of 10% vermiwash spray on 30 and 45 DAS recorded with the tallest plant height of 132.30 cm, followed by the treatment T_2- Foliar application of 3% panchagavya on 30 and 45 DAS with a plant height of 124.00 cm and it was found to be on par with the treatment T_9- Foliar application of DAP @ 2% + NAA @ 40 ppm spray on 30 and 45 DAS. The enhanced plant height observed in these treatments might be due to the application of recommended NPK besides the presence of macro, micro nutrients growth hormones, enzymes and vitamins in vermiwash. The least plant height of 81.00 cm was recorded in the control treatment (T_1). These results are in agreement with the research findings of Peeyush Kumar Jaysawal *et al*., (2020) [3] and Kajal Gill *et al*., (2018) [4].

Number of branches/plant

The number of branches/plant was significantly influenced by all the treatments. The treatment T_4- Foliar application of 10% vermiwash on 30 and 45 DAS resulted in the highest number of branches/plant (11.70). Vermiwash is the collection of excretory products and excess secretions of earthworms which had an excellent growth promoting compound as it was evident in terms of increased number of branches/plant. The treatment T_2- Foliar application of 3% panchagavya on 30 and 45 DAS and treatment T_9- Foliar application of DAP @ 2% + NAA @ 40 ppm spray on 30, 45 DAS exerted similar effect. Foliar application of panchagavya might have enhanced the biological efficacy of crops due to the presence of macronutrients, micronutrients and growth stimulants. Humic acid, fish meal extract, bokashi and polyfeed sprays were found to be superior than water spray and were comparable.The least number of branches/plant was recorded under the treatment T_1- water spray. These results are in accordance with the findings of Karur *et al*., (2015) [5] and Hatti *et al*., (2010) [6].

Number of leaves / plant

The number of leaves/plant was significantly influenced by various treatments tested. The treatment T_4- Foliar application of 10% vermiwash spray on 30 and 45 DAS resulted with the highest number of leaves/plant (142.70). The second best was T_2- Foliar application of 3% panchagavya on 30 and 45 DAS. Foliar application of DAP @ 2% + NAA @ 40 ppm and foliar application of polyfeed spray @ 1% were on par. The least number of leaves/plant of 76.00 was recorded under the treatment T_1- water spray. The vermiwash contains macro and micro nutrients, growth promoting substances and some metabolites. All these were enhanced the plant growth which was reflected in terms of increased leaf numbers. These results were in accordance with the findings of Deotale *et al*., (2019) [7] and Raman and Krishnamoorthy (2019) [8].

LAI

All the treatments significantly influenced the LAI. Among the treatments, T_4- Foliar application of 10% vermiwash spray on 30 and 45 DAS recorded with the highest LAI of 3.6. This might be due to the combined effect of basal application of nitrogen which is a component of amino acids, enzymes and hormones and foliar feeding with vermiwash. The next is order was T_2- Foliar application of 3% panchagavya on 30 and 45 DAS. Panchagavya is a organic fermented product contains growth promoting substances which stimulated the plant growth. Inorganic foliar sprays (DAP @ 2% + NAA @ 40 ppm and polyfeed @ 1%) exerted similar effect. The presence of nitrogen in DAP and in polyfeed rapidly penetrate the cuticle, enters the cell and facilitated the utilization of nutrients. Further, NAA auxin which might have also contributed for enhanced LAI. A comparable effect was observed between the treatments T_5- Foliar application of 1% Huminol Gold Plus (humic acid), T_6- Foliar application of 1% fish meal extract , T_7- Foliar application of 5% bokashi in influencing the LAI. This might be due to the presence of auxins in fish amino acid and in humic acid which stimulated the cell division and cell enlargement. The lowest LAI of 2.30 was recorded under the treatment T_1- water spray. The increased LAI due to the presence of nutrients and growth

promoting substances in vermiwash increased the seedling vigour and growth of plants. These results were in lined with the findings of Chattopadhyay (2015) [9] and Verma *et al*., (2017) [10].

Dry Matter Production

The DMP was influenced by all the treatments significantly. Various treatments increased the DMP from 96 to 270 kg/ha over water sprayed control. Among the treatments, foliar application of 3% panchagavya on 30 and 45 DAS recorded with the higher value of DMP (2028 kg/ha) and comparable with vermiwash spray and polyfeed spray. This might be due to taller plants with more number of branches and leaves observed in this treatment. Similar result was reported by Sonali Rajasooriya and Brindha Karunarathna (2020) [11]. The least DMP of 1758 kg/ha was noted in water sprayed control.

Seed yield

The seed yield was significantly differed due to various treatments. Various treatments increased the seed yield from 5.5 to 18.9 % compared to water sprayed control. As regards to foliar application of various organic and inorganic fertilizers, the highest seed yield of 821 kg/ha was recorded in the treatment T_4- Foliar application of 10% vermiwash on 30 and 45 DAS and it was followed by T_2- Foliar application of 3% panchagavya on 30 and 45 DAS. The enhanced yield in these treatments might be due to the presence of macro, micro nutrients, hormones and microorganisms which stimulated the growth attributes and it was reflected in terms of yield. The bokashi spray @ 5% and banana pseudostem extract spray @ 2 % increased the seed yield up to 10.3 and 5.5%, respectively over water sprayed control. The treatments T_9- Foliar application of DAP @ 2% + NAA @ 40 ppm on 30, 45 DAS and T_{10}- Foliar application of polyfeed (NPK 19:19:19) @ 1% on 30, 45 DAS were on par. Water sprayed control resulted in the lowest yield of 690 kg/ha. The similar findings were reported by Deotale *et al*., (2019).

Economics

The additional cost involved for foliar application of various organic and inorganic fertilizers were ranged from Rs. 400 to Rs. 10800 compared to water sprayed control. Among the treatments, the highest BCR of 2.51 was noticed in Vermiwash spray @ 10% and DAP @ 2% + NAA @ 40 ppm spray. An identical BCR value of 2.4 was noticed in Panchagavya spray @ 3%, Bokashi spray @ 5% and Polyfeed (NPK 19:19:19) spray @ 1%. The least BCR was noticed in Biovita (Sea weed exrtract) spray @ 1% which was due to the highest cost of Biovita.

CONCLUSION

From this experiment, it is concluded that foliar application of 10% vermiwash @ 30 and 45 DAS along with recommended dose of fertilizers recorded with the highest growth attributes and increased the yield up to 18.9 % compared to recommended dose of fertilizers with water spray.

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Table 1. Effect of different organic and inorganic liquid fertilizers on growth, yield and economics of sesamum

Treatments	Plant height at Harvest (cm)	No. of branches/ plant at Harvest	No. of leaves/ plant (50 DAS)	LAI (50 DAS)	DMP at Harvest (kg/ha)	Seed Yield (kg/ha)	Total cost of cultivation (Rs/ha)	BCR
T_1 - Water spray	81.00	7.30	76.00	2.3	1758	690	30978	2.24
T_2 - Panchagavya spray @ 3%	124.00	10.70	134.00	3.6	2028	802	32778	2.47
T_3 - Biovita (Sea weed extract) spray @ 1%	91.30	8.30	106.70	2.6	1854	740	41778	1.79
T_4 - Vermiwash spray @ 10%	132.30	11.70	142.70	3.6	2012	821	32978	2.51
T_5 - Huminol gold plus (Humic acid) spray @ 1%	94.00	8.70	110.00	3.0	1889	740	39478	1.89
T_6 - Fish meal extract spray @ 1 %	102.00	9.00	115.30	3.1	1927	753	32478	2.34
T_7 - Bokashi spray @ 5%	107.00	9.30	119.00	3.2	1936	761	31378	2.44
T_8 - Banana pseudostem extract spray @ 2%	89.00	8.00	103.30	2.6	1875	728	31478	2.33
T_9 - DAP @ 2% + NAA @ 40 ppm spray	120.00	10.00	128.00	3.5	1983	800	32573	2.48
T_{10} - Polyfeed (NPK 19:19:19) spray @ 1%	113.00	9.70	125.00	3.3	1961	785	32578	2.43
SEd	2.78	0.62	3.79	0.29	57.67	23.12		
CD (P=0.05)	5.85	1.30	7.96	0.61	115.35	48.66		

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RESEARCH ARTICLE

Field Evaluation of Different Formulations of *Azospirillum* and Phosphobacteria Inoculants on Maize Crop

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ABSTRACT

Field experiments were conducted to evaluate the performance different formulations of *Azospirillum and* phosphobacteriaon the growth and yield of maize var. Co 1. The experiment was laid out using Randomized Block Design (RBD) with three replications. The following treatments *viz*., T1 – Control, T2 – *Azospirillum* (log phase cells), T3 – *Azospirillum* (cyst), T4 – Phosphobacteria (log phase cells), T5 – Phosphobacteria (spore) and T6- Azophos (cyst and spore). The inoculation effect of different formulation on plant growth parameter like dry matter production and yield parameters viz.,grain weight g plant-1, cob weight g plant-1, 100 seeds weight (g), grain and stalk yield, N and P uptake by plants and finally rhizosphere population of *Azospirillum* and phosphobacteria were studied.It was observed that the treatment T6 (Azophos cyst and spore form) recorded a maximum growth and yield parameter followed by T3 (*Azospirillum* cyst). The results clearly indicated that cyst and spore forms of *Azospirillum* and Phosphobacterial inoculants resulted in maximum dry matter production and grain yield of maize when compared to the conventional form of *Azospirillum* (vegetative cells) inoculant. The inducement of cyst and spore resulted in better survival of organisms in the soil, resulting in maximum grain yield of maize.It was concluded that inoculation of Azophos (cyst form of *Azospirillum* and spore form of *Bacillus* sp.) formulation could augment the growth and yield parameters of maize by fixing higher amount atmospheric nitrogen, solubilization of phosphorous and secreting higher amount of plant growth promoting substances like Indole acetic acid (IAA) and Gibberellins.

Keywords: *Azospirillum, Bacillus* sp, Azophos, maize, Indole acetic acid, Gibberellins

INTRODUCTION

Biofertilizers are eco-friendly and environmentally safe and there is a growing awareness among the farmers about their use. Biofertilizers have been recognized as a vital component of the integrated nutrient supply system and organic farming (Martinez – Morales *et al.,* 2003). *Azospirillum* is one of the important biofertilizers, which is found to fix atmospheric nitrogen in association with crops like rice, maize, sorghum, wheat, and millets. Besides nitrogen fixation, *Azospirillum* secretes plant growth regulators viz., Indole - acetic acid, gibberellic acid, vitamins, etc. (Cassan *et al.,* 2009, Kumaresan *et al*., 2018). One of the vital problems in inoculant technology is the survival of the microorganisms during storage and several parameters have an influence on their survival viz., the culture medium. The physiological state of the microorganisms when harvested, the process of dehydration, rate of drying (Mary *et al*., 1985), the temperature of storage, and water activity of the inoculum. All these factors lead to the shorter shelf life of inoculants i.e., three to six months under normal storage conditions. Hence studies to increase the shelf life of inoculants or find alternate formulations for carrier inoculants are gaining importance.

MATERIALS AND METHODS

Source of *Azospirillum* and Phosphobacteria

Azospirillum and Phosphobacteria cultures were obtained from the Biofertilizer unit of Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, and Chidambaram.

Studies on the induction of encystment in *Azospirillum*

The *Azospirillum*culture was grown in N-free malic acid broth up to the OD value of 1.45 to get a population of 10^9 cells perml. The cells were harvested by centrifugation at 5000 rpm at 4°C and washed three times with 100 mM potassium phophate buffer solution. Twenty-five ml of this culture was inoculated into the minimal salts medium (Neyra and Dobereiner, 1977) and incubated at 120 rpm at room temperature.

Regeneration of cyst cells into vegetative cells

Cyst cells of A*zospirillum* was examined in N- free malic acid medium with ammonium chloride (0.0 I %) as nitrogen source (N + malate medium). Cyst inoculum was inoculated in to N+ malate medium and incubated at 120 rpm at room temperature. The morphological ranges of cyst cells were observed at hourly interval with phase contrast microscope. The regeneration of cyst cells were examined by serial dilution and plating up to 12 h.

Induction of sporulation in phosphobacteria (*Bacillus* sp.)

Sporulation was induced by supplementary nutrient medium (Setlow and Kornberg, 1969). This media was prepared as per the composition. After inoculation the cells were observed under phase contrast microscope at 24 h interval to calculated frequency of sporulation.

Regeneration of sporulated cells into vegetative cells

Regeneration of spores of *Bacillus* sp. was examined in nutrient broth. One ml of sporulated medium inoculated in nutrient broth and stored at room temperature. The regeneration and multiplication of cells were examined by serial dilution and plating technique (Allen, 1953) at 3 h interval up to 12h.

Evaluating the performance different formulations of bioinoculantson the growth and yield of maize var. Co 1:

To evaluate the performance different formulations of bioinoculants on the growth and yield of maize var. Co 1. The seeds were surface sterilized and inoculated with the standardized quantity of 20 g kg^{-1} of seed for different formulations *viz*., *Azospirillum* (log phase cells and cyst forms) and phosphobacteria (log phase of cells and spore).The field experiments were conducted in Randomized Block Design (RBD) with triplicates with the following treatments schedule

T1 - Control
T2 – *Azospirillum* (log phase cells)
T3 – *Azospirillum* (cyst)
T4 – Phosphobacteria (log phase cells)
T5 – Phosphobacteria (spore)
T6- Azophos (cyst and spore)

Observations on growth and yield parameters of maize

Five plants from each treatment were randomly selected for recording growth and yield parameters periodically at harvest.

Effect on dry matter production

Three plants from each plot were randomly selected, uprooted carefully and thoroughly washed on 30 DAS. Then the samples were dried in hot air oven at 80°C until a constant weight was obtained. The dry matter production was calculated and expressed in grams per plant.

Grain weight

The weight of grains from each cob of selected five plants in net plot was taken and expressed in g plant^{-1}.

Cob **weight**

The cobs from three randomly selected plants were removed thoroughly, air dried, cleaned and weighed. The average cob weight was taken as weight of cob ing plant^{-1}.

Hundred Seed weight

The weight of hundred grains were recorded from the samples drawn from the produce obtained in each of the net plot and is expressed in gram per hundred seeds.

Grain yield

At physiological maturity, cobs were harvested plot wise. The yield per hectare was calculated and expressed in tonnes per hectare on the basis of total weight of the cobs in net plot (kg ha^{-1}).

Stalk yield

After drying of stalk, the stalk yield for each net plot was recorded and yield per hectare was calculated.

Effect on total nitrogen content:

The total nitrogen content of the plant was estimated by Kjeldahl method (Humphries, 1956).

Studies on the survival of *Azospirillum* and Phosphobacteria population in rhizosphere soil by inoculation different bio inoculants in maize:

The number of *Azospirillum* and Phosphobacteria were determined by the most probable number technique (MPN) using Dobereiner medium and Pikovaskiya s medium during 30, 60, 90 DAS and at harvest stage.

RESULTS AND DISCUSSION

Conversion of vegetative cells of *Azospirillum* into cyst cells in Minimal Salt Medium (MSM)

Azospirillum cells were inoculated into Minimal Salt Medium (MSM) and the conversion of vegetative cells into cyst forms was observed from 12 h to 96 h. The results revealed that the cyst conversion was 11, 25, 53, 74, and 92 per cent for 12, 24, 48, 72, and 96 h respectively.

Regeneration of cyst cells of *Azospirillum* into vegetative cells in N + Malate medium

The population count of *Azospirillum* revealed that N + malate medium supported rapid regeneration and multiplication.

Induction of sporulation of *Bacillus* sp. in Supplementary Nutrient Medium (SNM)

Supplementary Nutrient Medium (SNM) induced the conversion of vegetative cells of *Bacillus* sp. into spore cells.

Regeneration of sporulated *Bacillus* sp. Culture:

The sporulated *Bacillus* sp. was known to regenerate in nutrient broth.

Effect of different forms of microbial inoculants on dry matter production, grain and straw yield of maize

The inoculation of Azophos influenced the crop growth and yield of maize. The dry matter production, grain weight g plant-1, cob weight g plant-1, 100 seeds weight (g) were found to be higher in Azophos (cyst and spore) inoculated plots than uninoculated plots (control). The dry matter production was recorded for different treatments, the treatments T 1 (control) and T 4 (Phosphobacterial log-phase cells) resulted in the lowest plant height whereas the treatment T6 (Azophos cyst and spore) and T3 (*Azospirillum* cyst) recorded the highest plant height i.e. the combination of cyst form of Azospirillum and spore form of Phosphobacteria resulted in maximum plant height, followed T3 (*Azospirillum* cyst). The results of the present study are similar to the finding of Watanabe and Lin (1984) who reported increased crop growth in maize inoculated with mixed cultures of *Azospirillum* and Pseudomonas sp.

The grain and straw yield of maize was recorded after the harvest of the crop. It was observed that the treatment T6 (Azophos cyst and spore form) recorded a maximum grain yield of 6.6 t ha-1 followed by T3 (*Azospirillum* cyst) 6.40 t ha-1. Whereas the treatment T2 (*Azospirillum* log-phase cells) recorded the grain yield of 6.21 t ha-1. The results clearly indicated that cyst and spore forms of *Azospirillum* and Phosphobacterial inoculants resulted in maximum grain yield of maize when compared to the conventional form of *Azospirillum* (vegetative cells) inoculant. The inducement of cyst and spore resulted in better survival of organisms in the soil, resulting in maximum grain yield of maize. Similar results were recorded in the haulm yield of maize. These investigations are in agreement with the findings of Thamizhvendan and Subramanian (2000) who observed increased rice yield due to combined inoculation of *Azospirillum* and Phosphobacteria.

Studies on the survival of *Azospirillum* and Phosphobacteria population in rhizosphere by inoculation different bio inoculants in maize

In the present investigations effect of different bio inoculants on the *Azospirillum* and phosphobacteria population in the rhizosphere was studied (Table-III). The T6-Azophos (cyst and spore cells of *Azospirillum* and Phosphobacteria) recorded a higher *Azospirillum* population, followed by T3 (*Azospirillum* cyst) and T2 (*Azospirillum* vegetative cells) at 60 DAS. poshobacterial population was showed maximum during 60 DAS. Among the treatments, T6 (Azophos cyst and spore) showed more number of phosphobacterial colony followed by T5 (Phosphobacterial spore) and T4 (Phosphobacterial vegetative cells). The application of phosphobacteria influenced the phosphobacterial population. Azophas (cyst and spore) and Phosphobacteria (spore) showed a higher population of *Azospirillum* and phosphobacteria in the maize rhizosphere when compared to other treatments.

Effect of different forms of microbial inoculants on plant N and P content of maize

The treatment T6-Azophos (cyst and spore cells of *Azospirillum* and Phosphobacteria) recorded maximum nitrogen uptake, followed by T3 (*Azospirillum* cyst) and T2 (*Azospirillum* vegetative cells). Plant phosphorus was recorded maximum during harvest. Among the treatments, T6 (Azophos cyst and spore) recorded the highest level of phosphorus followed by T5 (Phosphobacterial spore) and T4 (Phosphobacterial vegetative cells). Application of phosphobacteria enhanced the uptake of phosphorus and T6 (cyst and spore form of *Azospirillum* and Phosphobacteria) recorded the maximum available phosphorus indicating that phosphate solubilization was more in this treatment when compared to other treatments. Azophas (cyst and spore) and Phosphobacteria (spore) showed

higher nitrogen and phosphorus content in the maize plant when compared to other treatments. A similar result was reported by Belimov *et al.* (1995) who observed enhanced absorption of Nitrogen and Phosphorus in barley plants.

CONCLUSION

The inducement of cyst in *Azospirillum* and spore in *Bacillus* resulted in better survival of organisms in the soil, resulting in maximum growth and yield attributes of maize. It was concluded that inoculation of Azophos (cyst form of *Azospirillum* and spore form of *Bacillus* sp.) formulation could augment the growth and yield parameters of maize by fixing higher amount atmospheric nitrogen, solubilization of phosphorous and secreting higher amount of plant growth promoting substances like Indole acetic acid (IAA) and Gibberellins.

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Table I. Effect of different forms of microbial inoculants on of growth and yield components of maize.:

Treatments	Dry matter production (g plant $^{-1}$)	Grain weight per plant (g)	Cob weight per plant (g)	100 seeds Weight (g)	Grain yield (t/ha)	Stalk yield (t/ha)
T_1– Control	186.23	103.61	175.36	22.18	3.81	7.16
T_2 - *Azospirillum* (log phase cells)	255.00	142.74	270.06	26.00	6.21	10.24
T_3 – *Azospirillum* (cyst)	260.34	144.45	274.45	27.49	6.40	10.33
T_4 – Phosphobacteria (log phase cells)	251.63	139.24	265.56	25.10	6.01	9.86
T_5 – Phosphobacteria (spore)	252.75	141.13	269.16	27.53	6.20	10.16
T_6 Azophos (cyst and spore)	265.03	146.36	278.41	28.62	6.65	10.90
SEd	**0.647**	**0.834**	**1.463**	**0.451**	**0.081**	**0.040**
CD(p=0.05)	**1.321**	**1.677**	**2.967**	**0.936**	**0.165**	**0.084**

Table II. Effect of different forms of microbial inoculants on nitrogen and phosphorous uptake at different stages of growth in maize

Treatments	N uptake (g plant^{-1})			P uptake (g plant^{-1})		
	30 DAS	**60 DAS**	**Harvest**	**30 DAS**	**60 DAS**	**Harvest**
T_1– Control	0.189	0.562	1.378	0.119	0.362	1.078
T_2 - *Azospirillum* (log phase cells)	0.566	1.658	3.082	0.142	0.380	1.114
T_3 – *Azospirillum* (cyst)	0.579	1.685	3.124	0.155	0.413	1.136
T_4 – Phosphobacteria (log phase cells)	0.242	0.580	1.414	0.366	1.258	2.382
T_5 – Phosphobacteria (spore)	0.205	0.513	1.436	0.379	1.385	2.424
T_6 Azophos (cyst and spore)	0.484	1.491	2.900	0.284	0.991	2.300
SEd	**0.009**	**0.027**	**0.027**	**0.008**	**0.023**	**0.025**
CD(p=0.05)	**0.019**	**0.056**	**0.055**	**0.017**	**0.052**	**0.053**

Table III. Studies on the survival of *Azospirillum* and phosphobacteria population in rhizosphere by inoculation different bio inoculants in maize

Treatments	*Azospirillum* population (× 10^7 CFU g $^{-1}$ of soil)			Phosphobacterial population (× 10^7CFU g $^{-1}$ of soil)		
	30 DAS	**60 DAS**	**Harvest**	**30 DAS**	**60 DAS**	**Harvest**
T_1 - Control	0 .03	0.07	0.06	0 .02	0.05	0.04
T_2 - *Azospirillum* (log phase cells)	6.94	8.58	6.92	0.15	0.34	0.22
T_3 – *Azospirillum* (cyst)	7.35	9.99	7.40	0.25	0.35	0.23
T_4 – Phosphobacteria (log phase cells)	0.55	0.94	0.72	3.75	5.54	4.82
T_5 – Phosphobacteria (spore)	0.35	0.65	0.43	4.85	7.45	6.63
T_6-Azophos (cyst and spore)	4.72	5.98	4.68	2.94	4.58	3.92
SEd	**0.059**	**0.019**	**0.044**	**0.054**	**0.013**	**0.041**
CD(p=0.05)	**0.118**	**0.039**	**0.089**	**0.113**	**0.035**	**0.085**

RESEARCH ARTICLE

A Novel HPLC Method was Developed for the Estimation of Carbotegravir and Rilpivirine in Carbotegravir and Rilpivirine Pharmaceutical Dosage forms of Tablets and Drug Substance

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ABSTRACT

A simple, accurate, precise method was developed for the estimation of Carbotegravira and Rilpivirinein the tablet dosage form of Carbotegravira and Rilpivirine. Optimized separation was achieved on an Ascentis C18 150x 4.6mm, 5µusing mobile phase composition of phosphate buffer pH 4.0 and acetonitrile in the ratio of 700 mL:300 mL (v/v), at a flow rate of 1.0 mL/min, the injection volume is 10 µL and run time 6 minutes in isocratic elution. UV detection was carried out at a wavelength of 230 nm. The temperature was maintained at 30°C. Well-resolved peaks were observed with high numbers of theoretical plates, lower tailing factor, and reproducible relative retention time. The method was validated and all the validation parameters were found to be within the acceptable limits.

Keywords: Carbotegravir, Rilpirivine,stress degradation, RP-HPLC method development, Validation.

INTRODUCTION

Carbotegravira and Rilpivirine injectable suspension (trade name is CABENUVA), is indicated as a complete regimen for the treatment of human immunodeficiency virus type 1 (HIV-1) infection in adults to replace the current antiretroviral regimen in those who are virologically suppressed (HIV-1 RNA less than 50 copies per mL) on a stable antiretroviral regimen with no history of treatment failure and with no known or suspected resistance to either cabotegravir or rilpivirine [1,2,3,4]. A literature search confirms that there is no method reported for the simultaneous estimation of Carbotegravira and Rilpivirine quantitatively in Carbotegravira and Rilpivirine

Extended-Release injectable suspensions. Hence the present work aimed to develop simple stability indicating RP-HPLC method for the separation and quantification of Carbotegravira and Rilpivirine.The main aim of this method was to determine and validate the Carbotegravira and Rilpivirinebased on International Conference on Harmonization guidelines[5]. This method was made for a reproducible procedure for the quantitative analysis of drug samples as the bulk drug and in injectable suspensions. The designed method was considered advisable to develop a precise, accurate, simple RP-HPLC method. The chemical name for cabotegravir is (3S,11aR)-*N*-[(2,4-difluorophenyl)methyl]-6- hydroxy-3-methyl-5,7-dioxo-2,3,5,7,11,11a-hexahydro[1,3]oxazolo[3,2-*a*]pyrido[1,2-*d*] pyrazine-8- carboxamide. The empirical formula is $C_{19}H_{17}F_2N_3O_5$ and the molecular weight is 405.35 g/mol. It has the following structural formula: The chemical name for rilpivirine is 4-[[4-[[4-[(*E*)-2-cyanoethenyl]-2,6- dimethylphenyl] amino]-2-pyrimidinyl]amino] benzonitrile. Its molecular formula is C22H18N6 and its molecular weight is 366.42. Rilpivirine has the following structural formula:

EXPERIMENTAL

MATERIAL AND METHODS

Chemicals, reagents and instruments

Carbotegravira, Rilpivirine, Orthophosphoric acid (H3PO4), potassium dihydrogen phosphate (KH_2PO_4), Acetonitrile, and Milli-Q water. Ascentis C18 150x 4.6mm, 5µ column, HPLC instrument equipped with UV-VIS spectrophotometer & PDA detector.

Mobile phase and solutions preparation

Preparation of buffer:

Weigh and dissolve 1.36 g of potassium dihydrogen phosphate in 1000 mL of water, sonicate to degas, and adjust the pH of the solution to 4.0 with diluted orthophaspharic acid.

Preparation of Mobile Phase

Mix 700 mL of buffer and 300 mL of acetonitrile and sonicate to degas.

Preparation of Diluent:

Mix 500 mL of water and 500 mL of acetonitrile and sonicate to degas.

Chromatographic conditions:

Flow rate: 1.0 mL, Injection volume: 10 µL, Detector: 230 nm, column temperature: 30°C, Column: Ascentis C18 150x 4.6mm, 5µ, Run time: 6 minutes.

Standard Preparation

Accurately Weighed and transferred 45 mg of Carbotegravir and 2.5 mg of Rilpivirine working Standards into a 50 ml clean dry volumetric flasks, added 10 mL of diluent, sonicated for 10 minutes, and make up to the final volume with diluent. (900 µg/mL Carbotegravir and 50 µg/mL of Rilpivirine), 1mL from the above two stock solutions was taken into a 10mL volumetric flask and made up to 10mL. (90µg/ml Carbotegravir and 5 µg/mL of Rilpivirine).

Sample Preparation

Accurately weighed equivalent weight of one tablet combination powder sample transfer into a 100 mL volumetric flask, 50 mL of diluents was added and sonicated for 25 minutes, further, the volume was made up with diluent and filtered the solution through 0.45µm membrane filter (1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe).
0.5 mL of filtered sample stock solution was transferred to a 10 mL volumetric flask and made up with diluent. (90µg/ml Carbotegravir and 5 µg/mL of Rilpivirine)).

Degradation studies

Oxidation

Taken 1 ml of stock solution of 1800 µg/mL Carbotegravir and 100 µg/mL of Rilpivirine& 1 ml of 20% hydrogen peroxide (H2O2) was added separately. The resultant solution was kept for 60 min at 30°C. For the HPLC study, the resultant solution was diluted to obtain 90µg/ml Carbotegravir and 5 µg/mL of Rilpivirine), then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Acid Degradation Studies

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe& 1 ml of 2N hydrochloric acid was added separately. The resultant solution was refluxed for 30 min at 60°C and neutralized acid with an equivalent volume of sodium hydroxide solution. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoic acid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Alkali Degradation Studies

Taken 1 ml of stock solution of 1800 µg/mL Bempedoicacid and 100 µg/mL of Ezetimibe& 1 ml of 2N sodium hydroxide solution was added separately. The resultant solution was refluxed for 30 min at 60°C and neutralized the base with an equivalent volume of hydrochloric acid solution. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoicacid and 5 µg/mL of Ezetimibe, then 10 µL injection volumes was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Thermal Degradation Studies

Taken 1 ml of stock solution of 1800 µg/mL Bempedoic acid and 100 µg/mL of Ezetimibe & exposed the solution to heat at 105°C for 6 hours. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoicacid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

UV lightstudies

Taken 1 ml of stock solution of 1800 µg/mL Bempedoicacid and 100 µg/mL of Ezetimibe& exposed the solution to UV light by keeping into the chamber. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoicacid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

Neutral Degradation Studies

Taken 1 ml of stock solution of 1800 µg/mL Bempedoicacid and 100 µg/mL of Ezetimibe& 1 ml of water was added separately. The solution was refluxed for 6 hours at 60°C. For the HPLC study, the resultant solution was diluted to obtain 90 µg/mL Bempedoicacid and 5 µg/mL of Ezetimibe, then 10 µL injection volume was injected into the system and the chromatogram was recorded to assess the stability of the sample.

RESULTS AND DISCUSSIONS

The current study describes a new and simple, reliable, economic elution RP-HPLC-PDA method for the estimation of Bempedoicacid and Ezetimibe tablets dosage form. The forced degradation studies were conducted for the by using several degradation conditions like acidic, alkali, oxidation, thermal, UV, neutral conditions and the proposed method was effectively employed from the resolution of employed sample peaks. To our present knowledge, no such detailed and stability indicating method has been presented for this tablet dosage form. The developed method finished use of PDA as a tool for peak integrity and purity confirmation. Therefore the proposed study method can be used for the quantification of Benpezoicacid and Ezetimibe in the pharmaceutical dosage form. Finally, this

method was carefully validated; as a result, it can be suggested for routine analysis and for testing quality through stability studies of the drugs.

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Table 1: System suitability results for Bempedoic acid

Injections	Retention Time(min)	Area	USP Plate Count	USP Tailing factor
1	2.674	2959336	4401	1.74
2	2.720	2928213	4379	1.73
3	2.729	2959687	4514	1.75
4	2.729	2947298	4521	1.74
5	2.763	2943603	4380	1.75
Mean		2947627		
Std. Dev.		12997.6		
% RSD		0.4		

Table 2: System suitability results for Ezetimibe

Injections	Retention Time(min)	Area	USP Plate Count	USP Tailing factor
1	2.167	128176	3338	1.47
2	2.186	127721	3395	1.46
3	2.209	125518	3389	1.46
4	2.209	127156	3359	1.46
5	2.225	128591	3371	1.47
Mean		127432		
Std. Dev.		1195.8		
% RSD		0.9		

Table 3: Linearity concentration table

Linearity level	Bempedoic acid		Ezetimibe	
	Concentration (µg/mL)	Peak area	Concentration(µg/mL)	Peak area
25 %	22.5	717005	1.3	33620
50 %	45.0	1471191	2.5	70693
75 %	67.5	2227987	3.8	109222
100 %	90.0	2967170	5.0	139303
125%	112.5	3625113	6.3	172872
150 %	136.5	4320206	7.5	207140
Correlation coefficient	0.999		0.999	

Table 4: Method precision results

Method precision		
S. No	Bempedoic acid	Ezetimibe
1.	100.34	98.96
2.	99.61	101.36
3.	100.72	100.09
4.	101.29	101.30
5.	99.17	101.14
6.	100.74	100.15
Mean	100.31	100.50
S.D	0.79	0.95
%RSD	0.8	0.94

Table 5: Accuracy results

Accuracy (%Recovery)			
S. No	Recovery level	Bempedoic acid	Ezetimibe
1.	50%-1	99.0	99.36
2.	50%-2	99.2	99.12
3.	50%-3	100.7	99.76
4.	100%-1	100.5	98.22
5.	100%-2	99.4	100.31
6.	100%-3	99.7	99.13
7	150%-1	99.3	99.69
8	150%-2	100.9	101.42
9	150%-3	100.3	100.99

Table 6: Degradation results for bempedoic acid

Stress condition	% Amount remaining	% Amount degraded	Peak Purity	
			Purity Angle	Purity Threshold
Acid	95.00	5.00	0.260	0.478
Base	95.54	4.46	0.285	0.454

Oxidation	94.04	5.96	1.230	1.399
Thermal	96.87	3.13	0.266	0.474
UV	98.11	1.89	0.221	0.441
Neutral	99.28	0.72	0.224	0.448

Table 7: Degradation results for Ezetimibe

Stress condition	% Amount remaining	% Amount degraded	Peak Purity	
			Purity Angle	Purity Threshold
Acid	94.72	5.28	1.414	1.743
Base	94.98	5.02	1.192	1.445
Oxidation	94.43	5.57	4.076	4.171
Thermal	96.48	3.52	0.626	0.704
UV	97.64	2.36	1.078	1.390
Neutral	98.83	1.17	1.211	1.472

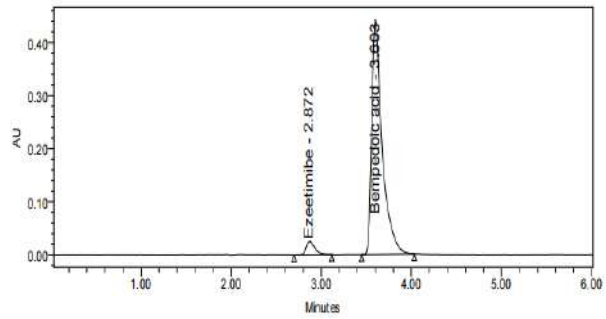

Figure 1: Standard chromatogram of Bempedoic acid and Ezetimibe

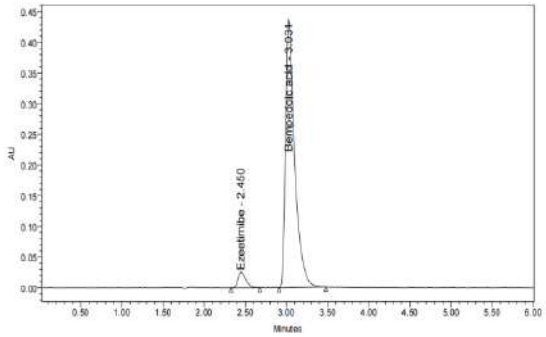

Figure 2: Sample chromatogram of Bempedoic acid and Ezetimibe

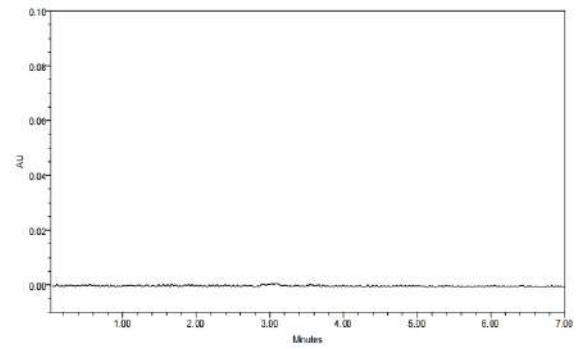

Figure 3: Blank chromatograms

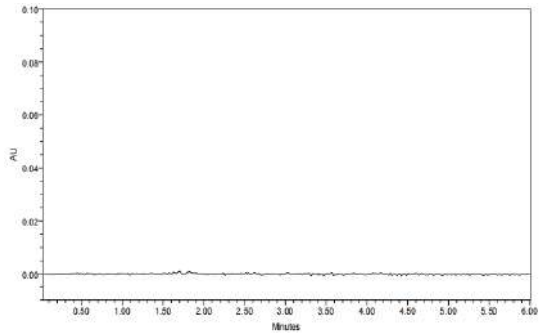

Figure 4: Placebo chromatogram

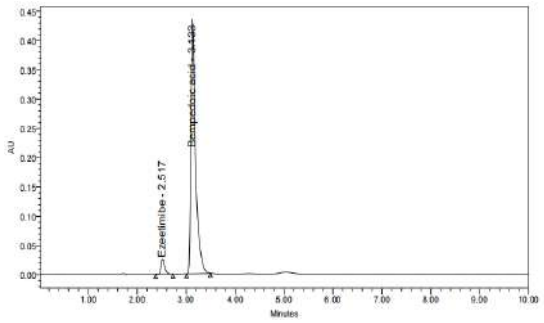

Figure 5: Acid degradation chromatogram

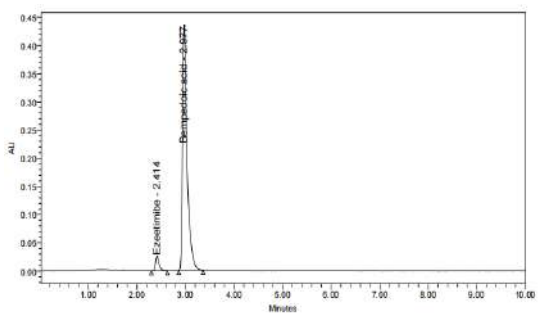

Figure 6: Base degradation chromatogram

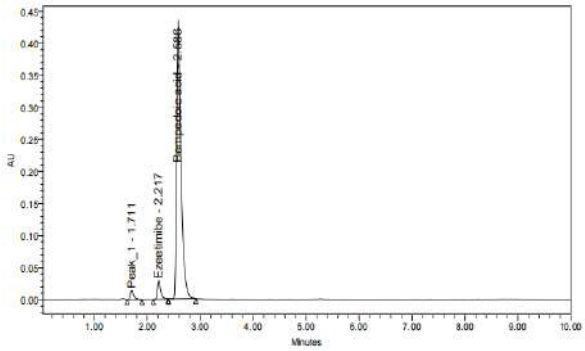

Figure 7: Oxidation degradation chromatogram

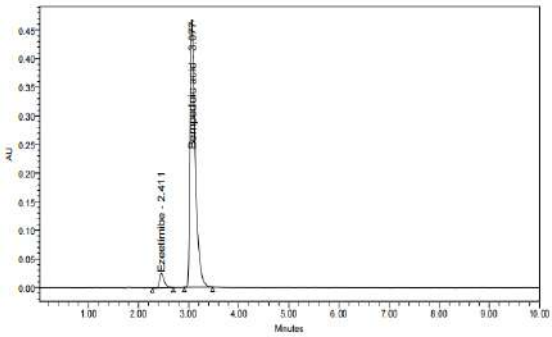

Figure 8: Thermal degradation chromatogram

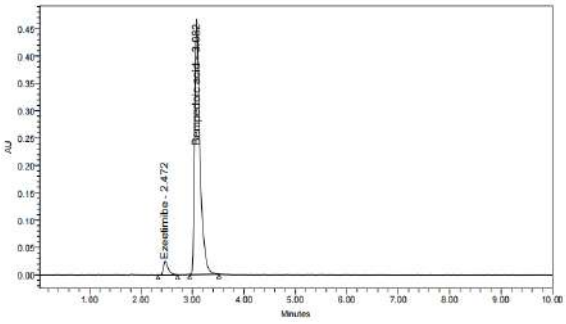

Figure 9: UV degradation chromatogram

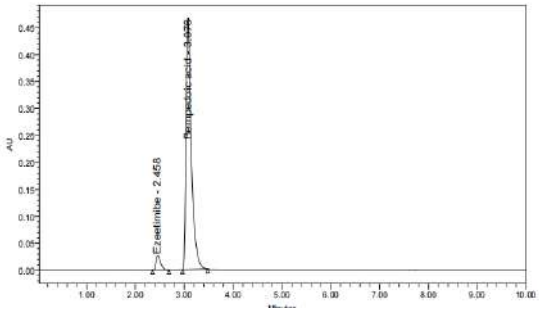

Figure 10: Water degradation chromatogram

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RESEARCH ARTICLE

An Efficient Trust Valuation Structure for Node Behavior Analysis in WSN

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ABSTRACT

The building of confidence in the Network Structure is critical for boosting cooperation and enhancing security. An appropriate trust estimation model should be created to successfully defend against attack and misbehavior to appropriately measure the trust relationships among nodes. A fresh quantitative model of trust value is proposed in this research. The Availability, security, honesty, privacy, integrity, and other trust variables connected to sensor node behaviors are all monitored. Each trust element is determined using the information entropy theory to eliminate the impact of the subjective setting. Our approach outperforms the competition in terms of thwarting attacks, according to the evaluation results.

Keywords: Node Behavior Analysis, WSN, Availability, Security, Privacy. Integrity, Honesty

INTRODUCTION

The behavior of nodes can be a significant component in determining node trust. The nodes can either "positively" or "negatively" activate. However, the source of this behavior could be real or fictitious to disrupt network reliability. This proposal is for a new node behavior prediction algorithm that estimates and forecasts a behavior category that may be employed in WSN for effective node trust management and reliable data transfer. The cooperative nature of nodes necessitates analyzing the behavior of trusted intermediate nodes to comply with privacy policies. In a WSN, routing is based on the collaboration and trustworthiness of intermediate nodes. It is critical to effectively manage the forwarding node for the successful completion of communication operations [1]. Each forwarding and target node acts within the network following its capabilities. To accomplish any communication operation, it decides their

actions and reactions independently. The behavior of a node can be deduced from its previous performance [2, 5]. Assume that a node performing a positive action has a negative history and that it will always behave adversely in a reliable pathway. Malicious nodes, on the other hand, can always prove their worth and keep the network stable for a long period. It only looks for positive action messages that are related to a given goal. It can use weighted trust mechanisms to calculate node trust. In this scenario, the node provides the prior actions a higher weighting than the current action. For safe pathways communication, the computed trust is employed to isolate hostile nodes from the network [3].

Types of Behavior

Nodes in wireless sensor networks (WSNs) can shift their behavior from cooperative to misbehaving at any time. According to [11], node behaviors in WSNs can be divided into four categories, as shown in fig 1. They can be categorized as follows:

- Cooperative Nodes: Which are involved in route finding and packet forwarding but not in attack initiation.
- In route discovery, failed nodes are not active.
- Malicious Nodes are involved in both route discovery and attack launch.
- Selfish Nodes perform route discovery but not packet forwarding. They tend to drop other people's data packets to save energy and send more of their packets, as well as to lower the latency of their packets.

The transition of Node Behavior

When a node joins the network, it is assumed to be cooperative or normal. It may alter its behavior to become a misbehaving node for a variety of reasons. Fig. 2 shows the transition of node behavior in WSNs. A cooperative node (C) can switch to a selfish, malevolent, or failed state. It is vulnerable to failure in a cooperative state due to energy fatigue, misconfiguration, and other factors. With the right setups, a selfish (S) node can be converted to a cooperative (C) node again. When a selfish or cooperative node is compromised or fails due to power depletion, it can become malignant. Even if a malicious (M) node becomes a failing (F) node, it will no longer be regarded as cooperative or selfish, even if its disruptive activities are just intermittent. The characteristics of a WSN in real-time can be changed at any time for a variety of reasons. As a result, node behavior in the real-time network can alter at any time. It could also be the result of some attacks or a lack of resource utilization required to keep network links and packet forwarding up and running. As a result, calculate the prediction of distinct categories' behavior with changes in the next behavioral observation.

- They can influence the reliability nodes owing to power loss and misinformation, which can lead them to fail, as well as other malicious attacks or selfishness that conserve resources.
- Proper reconfiguration can also help self-serving or malignant nodes regain their credibility. Due to a reduction in power supplies, this reconfiguration may be ineffective or fail.
- A malicious node can be a failed node, but it is no longer deemed unreliable or selfish if its harmful conduct is irregular.
- The node can be trusted again if the failing node's routing activity is stable regularly.

Behavioral changes are the most prevalent behavioral change found in a wide range of network circumstances, even though there is no explicit cause for them given the aforementioned assumptions. It reflects the power of privacy policies in WSNs to protect privacy. In WSNs, each node needs to forward packets from other nodes. However, because nodes in WSNs have resource restrictions, such as electric power, not all nodes are cooperative when it comes to packet forwarding. To save their resources, some selfish nodes do not forward packets from their neighbors.

Related Works

In [1] states throughput initially increases as network density grows, but after a certain point, say 50, it declines due to network congestion or delay. Because the number of packet drops increases as network density grows, PDR

lowers, delay generally increases, and energy usage decreases. A detailed assessment of the benefits and drawbacks of various routing methods is offered.

In [2] highlight the key research challenges and existing solutions in the realm of IoT security, as well as open topics and research suggestions for the future. The Internet of Things (IoT) is defined as a collection of disparate technologies that work together to deliver novel services across a variety of application domains. In this circumstance, ensuring that security and privacy needs are met is critical. In [3] offer a distributed and iterative centroid-based approach to solve the data aggregation challenges. The method selects two virtual anchors at each iteration and determines the order of a subset of nodes put between these two anchors. The proposed algorithm necessitates knowledge of local node connectivity as well as the identifier of the network's first sensor node, which is the only manually configured parameter.

In [4] by simulating smaller networks, propose a new method that uses statistical regression analysis to predict the energy consumption and lifetime of a wireless sensor network with hundreds or thousands of sensors. Also, a Revised LEACH technique was used to validate the suggested method. Indeed, this strategy can be used in different protocols and simulations to determine a given parameter's value. In [5] due to redundant node deployment and spatial and temporal correlations between sensed data, sensor readings typically exhibit both spatial and temporal redundancies. As a result, the distributed regression theory is employed in this paper to eliminate correlation in wireless multivariate monitoring sensor networks. Sensor nodes only send the regression model parameters, not data, to one another or the sink. The suggested technique minimizes the quantity of data and energy consumed during data transmission, hence extending the network's lifetime. Simulation is used to test the algorithm's energy usage and forecast accuracy to validate it. Simulation results suggest that the proposed technique is well suited to compressing multivariate monitoring data.

QoS Metrics

Performance measurements such as capacity, latency, response time, and loss probability are some of the characteristics of a system or network that must be understood and taken into account while modeling. Reliability, serviceability, and availability are examples of dependability metrics. Other performance or reliability (or both) indicators of interest include scalability, performance, cost, and so on [15]. In our work the following performance measures are used to determine the behavior: Availability, Security, Honesty, Privacy, and Integrity.

Availability

The possibility that a WSN node will function normally in the network is represented by its availability [9]. It is one of the most essential metrics for assessing the performance of a node. It is commonly stated in real-world computations as the probability of the available state when the network reaches a steady state. When a node reaches a stable state, its availability is determined by its energy and the environment in which it is located. Availability is a metric that expresses the likelihood that a component or system will perform at time t. instantaneous availability A refers to the availability at the time t. (t). The steady-state availability of a component is the proportion of time that it is operational. A = $\lim_{t\to\infty} A(t)$ is the formal definition (notice that this metric only makes sense in systems with a stationary probability condition [42]). Repair actions and availability are inextricably linked. In fact, following a failure, it is implied that the system is repaired; otherwise $\lim_{t\to\infty} A(t) = 0$. To calculate the instant availability of a host by using the probability of being in states 3, 4, and 5 as in equation (1):

$A(t) = 1 - \sum_{j=3}^{5} \phi_{ij}$ ----- ----------(1)

Security

One of the challenges in WSNs is to provide high-security requirements with constrained resources. The security requirements in WSNs are comprised of node authentication, data confidentiality, anti-compromise, and resilience against traffic analysis. To identify both trustworthy and unreliable nodes from security standpoints, the deployment

sensors must pass a node authentication examination by their corresponding manager nodes or cluster heads and unauthorized nodes can be isolated from WSNs during the node authentication procedure. Similarly, all the packets transmitted between a sensor and the manager node must be kept secret so that eavesdroppers cannot intercept, modify and analyze, and discover valuable information in WSNs. To measure the performance security level Encryption overheads are used. It has been discovered that different security techniques have varied impacts on the performance of wireless networks when they are configured within the network. The overheads associated with each security layer have been examined. The following is a breakdown of the overheads imposed by each security layer.
Total time spent by the sender and recipient processing the nth packet as it travels between them with security policy T (n, Px) represents Px, which is equivalent to

$$T(n,P_x) = T^s(n,P_x) + T^r(n,P_x) + T^t(n,P_x) \text{ ---------- (2)}$$

Where $T^s(n,P_x)$- is the time it takes a sender I with security policy P_x to process the nth packet. $T^r(n,P_x)$- is the time it takes a receiver j with security policy P_x to process the nth packet. $T^t(n,P_x)$ - is the time it takes the nth packet to travel between the sender and the receiver in the network with security policy P_x.
Security policies P_x and bit rate BR can be written as follows:

$$BR(P_x) = \frac{B_k}{\sum_{n=1}^{k}(T(n,P_x)) = \sum_{n=1}^{k}(T^s(n,P_x)+T^r(n,P_x)+T^t(n,P_x))} \text{ ---------- (3)}$$

Where $BR(P_x)$denotes the bit rate (bits/s), that can be obtained with each security policy(P_x).

$$BR(P_1) = \frac{B_k}{\sum_{n=1}^{k}(T(n,P_1)) = \sum_{n=1}^{k}(T^s(n,P_1)+T^r(n,P_1)+T^t(n,P_1))} \text{ ---------- (4)}$$

Where $BR(P_1)$ is the bit rate (bits/s), achieved by configuring the security policy with zero security level(P_1). Assume that$O(P_x)$ refers to the encryption overheads associated with various security policies, and that is defined as the difference between the bit rate for security layers and Overheads for encryption can be calculated as follows

$$O(P_x) = BR(P_x) - BR(P_1) \text{ ---------- (5)}$$

Honesty

A system's honesty, H (t), is the chance that it will operate without failure for a given time interval (0,t). While the end-user may believe the system is operating without errors, faults may be occurring within the system. Memory with Error Correction Codes is a nice illustration of this (ECC). A permanent problem may exist in memory in this instance, but the redundancy provided by ECC hides single-bit failures from the rest of the system. As a result, the following formula yields a host's honesty for the duration of t, denoted by H (t):

$$H(t) = 1 - \sum_{j=1}^{2} \phi_{j1} + \lambda \sum_{i=1}^{2} \phi_{i2} \text{ ---------- (6)}$$

Where λ is the penalty coefficient and $0 \leq \lambda \leq 1$
The difference between the total number of packets received at the destination and the total number of packets sent from the source. The number of packets per second received at the destination is measured by end-to-end network throughput. It is used as an external measure of a protocol's effectiveness in this case.

Privacy

WSN privacy is divided into two categories: context privacy and content privacy [18]. Context privacy is concerned with concealing the nodes' unique identities and locations, as well as the flow of communications transmitted within the network. An opponent can take advantage of the nodes' contextual information, such as their position. While content privacy is concerned with maintaining the messages' freshness, integrity, and non-repudiation, as well as their confidentiality. It is concerned with the data content that is exchanged within the network, which can be

compromised by data and traffic analysis attacks. In Privacy constraints, we use frame loss as measuring metrics. Frame Loss (FL) is a metric for data frame loss, defined as frames sent across a wireless network but not received at their destination. The loss of frames is calculated as follows

$$FL = \frac{Loads\,(Mbps) - Throug\,hput\ \ across\ \ the\ Load}{Load\,(Mbps)} \text{---------- (7)}$$

Integrity

Data gathered by sensor nodes may be sent to other middle nodes, requiring just minor calculations from data collecting to base stations. Integrity necessitates the transmission of accurate and true data. In this meaning, integrity refers to result integrity, with the final results received by base stations consisting of real results, excluding those having deliberately twisted and erased information. If attackers fabricate a data item, the sink can detect it without knowing the keys when sensor nodes deliver encrypted data that satisfy a query range. As a result, given the following three conditions, this article performs integrity analysis on decoded data. The loss of a bucket, if data from a bucket's dimension is missing, the sink can quickly locate it by comparing the query request and queries. After comparing the decoded and query, the actual received query request can be determined as having lost the data in the bucket. Network Life (NL) is defined as the amount of time until the message loss rate reaches a certain level. "Time to network partition" [10] is a more comprehensive term for the network's lifetime. When there is a cut-set in the network, the network partitions. It will be implemented as a new statistic based on energy variance

$$NL = E - (\underline{U} + \sigma) \text{ ---------- (8)}$$

Where$\underline{U} = \frac{\sum U_i}{N}$, E is the total initial energy at each node, U_i is the average used energy, N is the total number of nodes in the network, and σ is expressed as

$$\sigma^2 = \frac{(U_i - \underline{U})^2}{N} \text{ ---------- (9)}$$

All of these metrics are derived using their cumulative average values, which means that the performance value at time t is the average of the performance values from 0 to t. (seconds).

RESULT AND DISCUSSION

Using the above parameters to understand the node behavior in WSN. From Figure 3., it is stated that availability will be estimated based on the no of nodes and their states. The node availability is medium when the no of the node is modest; when the no of the node is large the node availability is high. Figure 4, denotes varying traffic with encryption overheads to understand the possible security policies to work on the bit rate in WLAN. When no of nodes are low the overheads related to security policies with bit rate is high as well no of nodes high it ultimately getting lower. From Figure 5, it is stated that honesty will be estimated based on the no of nodes and their effectiveness. The node honesty is low when the no of the node is low, and also when the no of the node is large the node. Honesty is high when it is modest. From Figure 6, it is clear that privacy will be estimated based on the no of nodes and their frame loss. The node frame loss is low when the no of the node is low. But comparatively frame loss is high when the node is modest or high. From Figure 7, it is clear that integrity will be estimated based on the network life and energy level of nodes. The node energy level optimizes to hold the message packets at a certain time duration when the no of the node is moderate. But comparatively, the lifetime is less when the node is high.

CONCLUSION

The study provided a formulation model for node activity detection in Wireless Sensor Networks. The model essentially demonstrates the flexibility of employing metrics to determine node behavior. Furthermore, metrics used here can deal with the uncertainties in a wireless sensor network. In a routing operation between a source and a destination, the model examines the activity of neighboring nodes. As a result, the network's performance is heavily reliant on the performance of nodes in a cluster, which is reflected by the level of cooperation among intermediary nodes. Availability, Honesty, Security, Privacy, and Integrity can be used to control the behavior of nodes in this paradigm.

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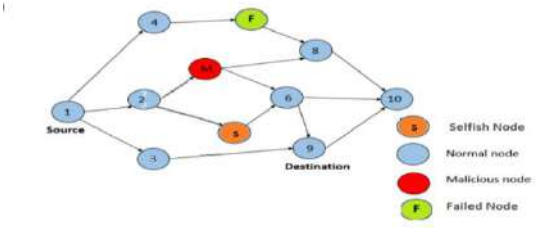

Figure: 1. Types of Nodes in WSN

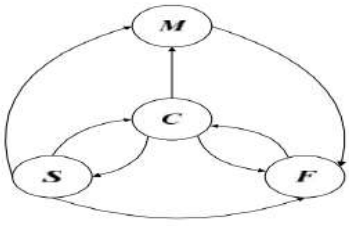

Figure: 2. The transition of Node Behavior

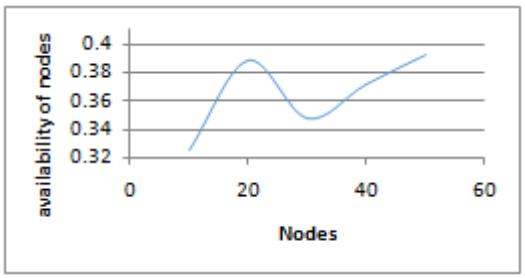

Figure 3. Availability of Nodes

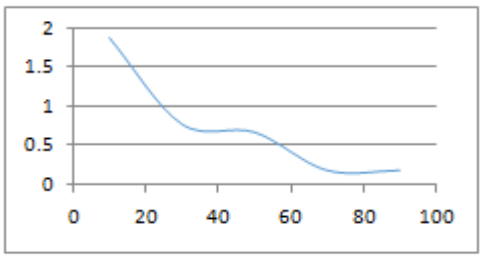

Figure 4. Encryption Overheads of Nodes

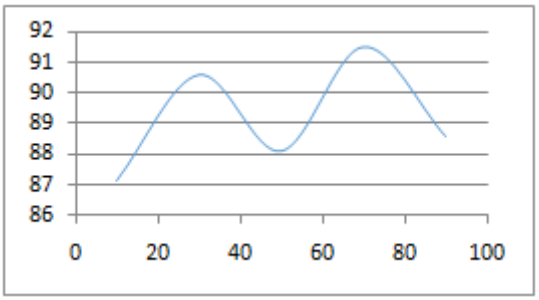

Figure 5. Honesty of Nodes

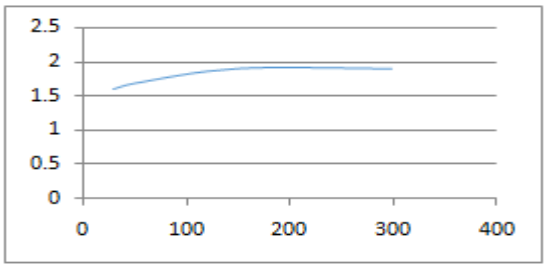

Figure 6. Frame Loss of Nodes

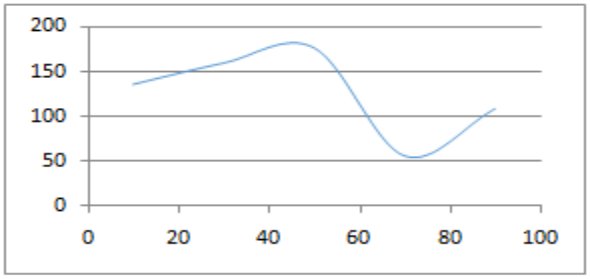

Figure 7. Network Life

REVIEW ARTICLE

Hyperlipidemia: Present Advances and Herbal Interventions

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ABSTRACT

The disorder known as hyperlipidemia occurs when the blood's level of triglyceride- or cholesterol-carrying lipoproteins exceeds a set normal threshold. It's a rather frequent ailment and a prominent component of the metabolic syndrome, which is bring about by number of variables. Cardiovascular complications are now well recognised as major causes of morbidity and mortality. When combined with other common disorders like hypertension, cardiovascular disease, diabetes, and so on, it becomes more worsen. There are several synthetic medications available nowadays, and the majority of them use to act towards hyperlipidemia have negative side effects that diminish the patient's quality of life. Because epidemiological evidence suggests that alternative treatments, particularly utilisation of herbal interventions and their supplements, can effectively treat hyperlipidemia, the trend toward consuming lipid-lowering medicinal plants has grown. The focus of the review paper is on Present Advances and Latest Natural therapies (herbal intervention) in hyperlipidemia.

Keywords: Hyperlipidemia, Antihyperlipidemic, Hypercholesterolemia, Bempedoic acid, Simvastatin, Molecular docking.

INTRODUCTION

Hyperlipidemia is a condition in which the blood has unusually high-caliber of lipoproteins and/or lipids due to improper metabolism.[1]. Excess lipids or fatty molecules in the blood cause it, and it is a major consequence for atherosclerosis and heart disease. Cholesterol, triglycerides, and lipoproteins, which are fat and cholesterol

molecules connected to protein, are all types of lipids found in the blood. Lipoproteins are classified as VLDL (very low-density lipoproteins), LDL (low-density lipoproteins), and IDL (intermediate-density lipoproteins) (IDL). Triglycerides, cholesterol, and protein make up chylomicrons, which are also known as lipoproteins. High density lipoproteins (HDL) are sometimes known as "antirisk" factors since they are inversely associated to heart disease risk.[2]

Hyperlipidemia in general can be divided into two subcategories:

1. Hypercholesterolemia, characterised by a high amount of cholesterol.
2. Hypertriglyceridemia, characterised by high amount of triglycerides, the most prevalent form of fat [3].

Types of hyperlipidemia

It can be categorized into primary and secondary hyperlipidemia.

Primary hyperlipidemia

It is usually due to genetic causes (such as a mutation in a receptor protein).

a) A single gene defect, is familial, and called 'monogenic' or genetic.

b) Multiple genetic, dietary and physical activity related causes: 'polygenic' or multifactorial [4].

Secondary hyperlipidemia

It's associated to diabetes, myxoedema, and persistent alcoholism, also the use of corticosteroids, oral contraceptives, and beta-blockers. Secondary hyperlipidemia results from a lack of lipoprotein or its receptor, which can be caused by diabetes, hypothyroidism, chronic renal failure, obesity, alcohol, and other disorders. These factors aggravate the condition of someone with primary hyperlipidemia.

Secondary hyperlipidemia is divided into four categories

(i) Hypercholesterolemia
(ii) Low HDL
(iii) Hypertriglyceridemia
(iv) Hypocholesterolemia.[5]

Present Advances in Hyperlipidemia

Bempedoic acid in hyperlipidemia

Current Management

Bempedoic acid (ESP-55016 and later ETC-1002) was the subject of numerous animal research investigating its effect in dyslipidemia. It was first identified as -hydroxy-alkanedicarboxilyc acid (ESP 55016) in female Zucker (fa/fa) rats, which had a lipid-lowering effect. Serum non-high-density lipoprotein (HDL) cholesterol, triglyceride, and non-esterified fatty acids were all reduced with ESP 55016. Serum HDL cholesterol levels increased in a dose-dependent manner, while serum glucose and insulin level decreased. This medication inhibited lipid production in rat hepatocytes. [21]

Mechanism of action

ETC-1002, a new drug intended to treat cardio-metabolic disorders and dyslipidemia, was discovered as a result of this mode of action. This chemical has a Ca (2+)/calmodulin-dependent kinase-independent and hepatic kinase 1-dependent effect on AMP-activated protein kinase. It inhibits ACLY and generates a CoA thioester in the liver, resulting in a decrease in cholesterol production [22].

Antihyperlipidemia Molecular Docking of Anthocyanin Compounds against PPAR, HMG-COA Reductase and ACAT proteins

The molecular mechanism of the anthocyanin effect of lipid lowering effect is unknown, so there is a need of molecular docking technique employing by in silico investigation. At Molecular level it allows to simulate receptor protein and their interconnection with ligands. The study used molecular docking strategies to inspect the achievable

of anthocyanin like anti-hyperlipidemia drug with the aid of inhibiting the PPAR protein, HMG-CoA reductase, and ACAT in silico. The molecular docking data were examined, the usage of the affinity electricity cost (kcal/mol) and the complex form between the receptor and the ligand. The anthocyanin compound received docking range of -8.8 kcal/mol, -6.0 kcal/mol, and -7.6 kcal/mol for the PPAR-protein, HMG Co-A reductase and ACAT proteins, respectively. This value is equal to Simvastatin's high quality influence docking range of -7.8 kcal/mol, -6.4 kcal/ mol, and -7.7 kcal/ mol. In silico, anthocyanin compounds can act as anti-hyperlipidemic marketers and inhibit the PPAR-protein, HMG Co-A reductase, and ACAT.[23]

For Primary hyperlipidemia, new treatments

New lipid lowering medications have received approval from the European Medicines Agency and US Food and Drug Administration. These medications target metabolic pathways like 5 triphosphates)-citrate lyase (bempedic acid), proprotein convertase subtilisin / kexin 9 (inclisiran), apolipoprotein CIII (volanesorsen), angiopoietin-like 3 (volanesorsen) . These have cumulative effects with the presently applicable remedy (i.e. statins, ezetimibe or fibrates). The addition of these cutting-edge medications to the treatment options for individuals with primary hyperlipidemia may increase their likelihood of achieving their therapeutic objectives. It might potentially be a safer option for people who are encountering side effects from already accessible medications [24].

A functional food used to reduce hyperlipidemia: Red yeast Rice

Statins may be substituted with red yeast rice (RYR), a traditional remedy and nutritious food with a long history. Small doses of RYR (equivalent to 5–7 mg of lovastatin daily) have been demonstrated to lower serum cholesterol as effectively as a 20–40 mg dose of pure lovastatin alone, suggesting that RYR contains bioactive compounds that lower cholesterol.[25]

In hamsters and mice, nitazoxanide-based anthelmintics guard against experimental hyperlipidemia and hepatic steatosis

Lipid metabolism problems results in hyperlipidemia and hepatic steatosis, for improving this problem at the same time it is optimal to create medications. Nitazoxanide is an oral antiprotozoal medication approved by the FDA, having good pharmacokinetic and safety profile. Tizoxanide, metabolites of nitazoxanide caused modest mitochondrial uncoupling and then activated AMPK in HepG2 cells. In hamsters, nitazoxanide was given as a gavage to prevent high-fat diet (HFD)-induced increases in liver weight, blood, and liver lipids, as well as to alleviate HFD-induced renal lipid accumulation. Nitazoxanide greatly reduced the histopathologic alterations in hamster livers caused by the HFD. Nitazoxanide had a therapeutic effect in hamsters through preceding hyperlipidemia and hepatic steatosis. HFD-induced hepatic steatosis in C57BL/6J mice was alleviated by gavage administration of nitazoxanide, as was WD-induced hepatic steatosis in Apoe–/– mice. According to the findings, repurposing nitazoxanide could be beneficial.[26]

The Effects of Quisqualis indica Methanolic Extracts on Passive Smoking-Induced Hyperlipidemia in Rats

In this Rats with passive smoking induced hyperlipidemia were treated with methanolic and aqueous extract of aerial components, mostly flowers. It was generated in a airtight enclosure incorporate one burning cigarette inside. By measuring the level of blood serum in UV at 505 nm following treatment with the reagent supplied in the auto span diagnostic kit, the hypolipidemic activity was determined. A dosage of 100 mg/kg p.o was generated using distilled water. At varied concentrations and doses, both QI extracts in blood serum significantly reduced the hazardous lipid layer, demonstrating that the plant has hypolipidemic properties. It significantly reduces LDL, VLDL, cholesterol, triglycerides, and elevates HDL levels in blood serum, which could be due to the plants antioxidants suppressing lipid peroxidation. The results reveal that methanolic plants extracts are more effective than non-methanolic plants extracts.[27]

Hyperlipidemia in post-COVID patients: a novel observational follow-up study

Alteration in plasma lipids levels associated with disease severity due to various viruses (bacteria, viruses). Studies show that lipid metabolism changes in patients hospitalized COVID-19 during and after (after COVID) disease.

Patients who were hospitalized in the wards of COVID-19 between April 02, 2020, and November 20, 2020 were examined at a non-follow-up treatment clinic and re-searched. Both during and after the COVID treatment, dyslipidemia was noticed in COVID-19 patients. The findings also support the evidence that low levels of LDL-C and / or HDL-C may rise the chance of serious infections, as well as COVID-19. Varying of lipid profile before / during and after the entire course of the disease should be noted.[28]

RNAi therapy for hyperlipidemia using thermostable, ionizable, lipid-like nanoparticles (iLAND)

Small interfering RNA (siRNA) is considered a promising treatment for hyperlipidemia. Advancement of siRNA drugs, creating a thermostable clinic delivery mechanism continues to be extremely difficult. Here, a series of substances such as ionzable lipids were logically designed; The 4 lipid composition panels are designed and tested according to four independent frames. At 40 ° C lead lipid (A1-D1-5) was stable, and the prepared formulation (iLAND) showed volume and dual genetic detoxification pattern with an average of 0.18 mg / kg of active dose. Serum cholesterol and triglyceride levels show strong reduction which can be achieved by delivering angiopoietin-like 3 or apolipoprotein C3 (APOC3) in high-fat diet-fed mices, db / db mice, and human APOC3 transgenic mice, respectively, compatible with displaying appropriate safety profiles. Thus, siRNA@iLAND prepared by thermostable A1-D1-5 shows a high rate of siRNA delivery, treatment for hyperlipidemia, and preventing later metabolic disorders [29].

Oxidative stress in hepG2 cells and hyperlipidemic rats reduced by Simvastatin

Simvastatin is a well-known anti-hyperlipidemic medication, although only a few studies have suggested that it can help reduce oxidative stress. Simvastatin was studied in vitro and in vivo for its molecular binding to numerous antioxidant enzymes, as well as the levels of these enzymes after treatment with simvastatin. This was the first study to show that simvastatin bind to catalase molecularly. Furthermore, the anti-oxidative activities not investigated in HepG2 cells exposed to Lipopolysaccharide (LPS)-induced oxidative stress. It successfully reduced oxidative stress by raising antioxidant enzyme levels. After the treatment with simvastatin (10 M, 24 h), the action of superoxide dismutase and catalase both considerably enhanced [30].

Hyperlipidemia Management with a Pravastatin-Loaded Nanogel

PS (pravastatin sodium) is a cholesterol-lowering medication. The goal of the study was to produce a Pravastatin-loaded nanogel and test its efficacy in treating hyperlipidemia. The ionic gelation process was used to prepare pravastatin-loaded chitosan nanoparticles (PS-CS-NPs), which were subsequently transformed to nanogel with the addition of specified quantity of 5% poloxamer solution. The designed and optimised formulation was tested on a number of criteria, including drug entrapment efficacy, in vitro drug release, and hemolytic activity. The nanogel formulation's in vitro drug release indicated the drug's sustained release (59.63 percent in 24 hours). According to the drug excipient compatibility studies, no contiguity between drug and screened excipients. The efficacy of drug entrapment was shown to be higher. In nanoformulation, the hemolytic action less hazardous than pure drug solution. The findings suggest that it as an active and cautious nano delivery method in the treatment of hyperlipidemia [31].

CONCLUSION

Hyperlipidemia, the most prevalent form of dyslipidemia, major cause of cardiovascular disease. It is marked by high-caliber of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) in peripheral blood, as well as low level of high-density lipoprotein cholesterol (HDL-C). It leads to deficiency in lipid metabolism on the surface of Apoprotein C-II or a malfunction in lipoprotein lipase activity. Genetic, nutritional, and environmental variables have all been linked to it. The treatment of hyperlipidemia usually entails a steady reduction in lipid levels using a variety of medications such as statins, fibrates, bile acid sequestrates and niacin. However, due to their severe side effects, the ramifications of these medications are debatable, according to this comprehensive assessment. Herbal extracts comprising selected portions of medicinal plants are employed intact or

their extracts including specific phytochemicals to normalizing the lipid in blood level and there are several current advances or development in hyperlipidemia.

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Table1:Primary hyperlipidemia can be further categorized into different types

Type	Synonyms	Deformity of serum	Imperfection
Type-I	Familial hyperchylomicronemia	Increase Chylomicron	Low LDL, Altered Apo C-II
Type-IIa	Familial hypercholesterolemia	Increase LDL	Decrease LDL receptor
Type-IIb	Familial combined hypercholesterolemia	Increase LDL & VLDL	Decrease LDL receptor & increase Apo B
Type-III	Familial dysbetalipoproteinemia	Increase IDL	Apo E2 synthesis defect
Type-IV	Familial hyperbetalipoproteinemia	Increase VLDL	Increase VLDL production, decrease Elimination
Type-V	Endogenous hypertriglyceridemia	Increase VLDL & Chylomicron	Increase VLDL production, decrease LPL

Table2. Antihyperlipidemic activity (Lipid lowering effect) of Latest Medicinal plants

SN	Plants (Common Name)	Family	Parts Used	Model Used	Dose(mg/kg)	Findings
1	*Camellia Sinensis* (Green Tea)	Theaceae	Leaves	Triton WR-1339	200	Aqueous extract of leaves was more effective to manage hyperlipidemia. Existence of polyphenolic compounds show hypolipidemic activity.[6]
2	*Elaeocarpus ganitrus* (Rudraksha)	Elaeocarpaceae	seeds	Cholesterol diet induced	250, 500	Ethanolic extract of seed exhibited cardio protective activity in rabbits. The strong presence of flavonoids might support to antihypercholester-lemic effect.[7]
3.	*Stellaria media* (Chickweed)	Caryoph-yllaceae	leaves	Diet induced, Triton induced	200, 400	The Plant posses good antihyperlipidemic activity in atherogenic diet induced hyperlipidemic rats & led to development of new herbal formulation possesses antihyperlipidemic & antiatheros-clerotic activities.[8]
4.	*Cassia tora* (Chakunda, Chakvat)	Caesalpiniaceae	Leaves	Cholesterol diet induced	100, 200	The flavonoid of leaves of Cassis tora show antihyperlipidemic activity in the heart of rats [9].
5.	*Ulva pertusa* (Green seaweed)	Ulvaceae	Whole (algae)	Cholesterol diet induced	125	Nicotynl ulva (NU) derivative from *U.pertusa* posses antihyperlipidemic activity. It's not only treat hypolipidemic condition but also prevent cardio-vascular and cerebero-vascular disease caused by hyperlipidemia.[10]
6.	*Catharanthus roseus* (Sadabahar)	Apocynaceae	leaves	Cholesterol Powder (400 mg/kg)	200	Catharanthus roseus leaf ethanolic extract has a considerable antihyper-lipidemic action. When combined with ator-vastatin, the antihyper-lipidemic benefits of the leaves ethanolic extract were not increased.[11]
7.	*Citrullus lanatus* (Water melon)	Cucurbitaceae	Seeds	High cholesterol	800	The plant significantly (P & lt; 0.05) and non-significantly

				diet & Triton X 100		(P & gt; 0.05) decreased the levels of total cholesterol, TGs, HDL, LDL, VLDL, asparate aminotransferase and alkaline aminotrans-ferase. Level of HDL maintain to normal range. It may be supportive treatment in combating hyperlipidaemias [12].
8	Gugulipid-*Commiphora mukul* (guggul)	Burseraceae	Dry guggul resin	Triton WR 1339 induced	6.75	Gugulipid demonstrated strong antihyperlipidemic efficacy and was proven to be most effective and safe when combined with atorvastatin at even lower doses [13].
9	*Spondias mombin* L. (Hog Plum)	Anarcadiaceae	Leaves	Streptozo-tacin induced	125, 250, 500	Diabetes-induced hyperlipidemia is responsible for excess movement of fat from adipose due to limited usage of glucose. MESM treatment decreased TC, TG, LDL, and VLDL levels while increasing HDL levels, implying that the methanolic extract of the plant may have hypolipidemic effect [14].
10	Sijukkot-*Lactuca indica* (Indian Lettuce)	Asteraceae (Compositae)	Leaves	High Fat diet	200, 300, 400	Flavonoids, tannins, saponins, steroids, and triterpenoids are detected in the ethanolic extract, having a beneficial effect on cholesterol levels in blood lipid profile of HDL and LDL [15].
11	*Oroxylum indicum* L. (Putivriksha, Bhutvriksha)	Bignonia-ceae	Fruits	High fat diet	100, 200, 300	The fruit extract has hypolipidemic effect in hyperlipidemic mice, and the active ingredients are flavonoids and volatile oils, explored as antihyperlipidemic agent.[16]
12	*Erythrina senegalensis* (Senegal coraltree)	Fabaceae	Leaves	Poloxamer 407-induced	200	Having polyphenols such as flavonoids, tannins, and other associated phytochemicals may explain the hypolipidemic effect of *Erythrina senegalensis* methanol extract. The MLEES

						decreased TC, TG and LDL by 37.49%, 57.54% and 34.58% respectively while it increased serum HDL by 32.50%.[17]
13	*Anogeissus leiocarpus* (African birch)	Combretaceae	Roots	60% Fructose overload	500	The polyphenolic components in the hydroalcoholic root extract of A. leiocarpus have demonstrated remarkable and powerful antihyperlipidemic and antioxidant effects [18].
14	*Allium cepa L.* (Red onion)	Amarylli-daceae	Bulb	Poloxamer 407 induced	200, 300, 400	Red onion ethanolic extract show dose dependent activities on body weight, serum lipid and atherogenic index. At the dose of 400 mg/kg better activities revealed [19].
15	*Carica papaya* linn. (Papaya)	Caricaceae	Leaves	High cholesterol diet	100, 200	The findings showed that the microwave-facilitate aqueous ethanol extract restored the hyperlipidemic impact at both 100 and 200 mg/kg per day, decreased atherogenic index and maintained lipid profile to reference levels because of the existence of polyphenols and flavonoids.[20]

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RESEARCH ARTICLE

Efficacy of Cow Urine Based Derivatives Combination with RDF as Plant Growth and Enhance Yield on Paddy Crop

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ABSTRACT

The field experiment was conducted during 2018-2019 at Rajagoplapuram village in farmer's holding, Mayiladuthurai district, Tamil nadu, to study the growth, yield attributes and yield of rice as influenced by foliar spraying of cow urine based derivatives in combination with RDF in sandy loam soil. The experiment was laid out in randomized block design. The experiment consisted seven treatments. T1- NPK (RDF + water spray), T2- NPK + foliar spray of CUD @5% (SS), T3- NPK + foliar spray of CUD @10% (SS), T4- NPK + foliar spray of CUD@ 15% (SS), T5- NPK + foliar spray of CUD @5% (DS), T6- NPK + foliar spray of CUD @10% (DS), T7- NPK + foliar spray of CUD @15% (DS). The 100 per cent recommended dose of inorganic NPK fertilizers were applied uniformly to all plots. The results revealed that foliar application of cow urine based derivatives as single or double spray significantly increased the growth attributes, yield attributes and yield of rice. Regarding growth attributes *viz*., plant height, number of tillers, leaf length, leaf breadth and yield attributes *viz.*, number of paniclesm^{-2}, number of grains panicle^{-1}, panicle length and number of filled grains panicle^{-1} and the maximum values were recorded with foliar application of 10% cow urine based derivatives attillering stage but this was found to be on par with 15% single and double spray of CUD. The maximum grain yield 3917 kg ha^{-1} was recorded with foliar application of 10% (Single spray) of CUD and significantly superior to rest of the treatments. The control (RDF+waterspray) registered lowest grain yield of 1883 kgha^{-1}.

Keywords: Cow urine based derivatives, inorganic NPK fertilizers, Growth, grain yield, rice)

INTRODUCTION

Rice (*Oryzae sativa* L.), is a staple food crop in India. Rice play vital role in food security and it is means of livelihood of millions of people making a slogan "Rice is life" most appropriate. Globally, the highest area under rice in India (43.86 million hectare) and stood second position in production (104.80 million tons) after china (144.85 million tons) with an average productivity of 3.77 t ha^{-1} [1].Within the country, rice occupies one quarter of the total cropped area, which contributes about 40-43 per cent of total food grain produced; is a source of livelihood for millions of Indians and also earn foreign exchange worth 12,000 crores . The fourth largest rice producing state in the country is Tamil Nadu which produced about 7.98 million tonnes of rice during 2015-2016. The area on which rice was cultivated in the state amounted to 2.04 million hectares. The national food security heavily depends on rice. India is primarily agrarian, and this sector provides livelihood to a major part of the population. To feed the 1.3 billion population of the country, increasing tremendously approximately at 1.2% every year, the food grain production need to be increased correspondingly, towards attaining this goal, there is requirement of higher doses of fertilizers, which require non-renewable energy. In the same time, declining factor productivity owing to imbalanced and indiscriminate use of fertilizers in most productive zone of the country i.e. Indo Gangetic Plains, food grain production in India reached to the plateau. In recent days farmers facing many problems like – insufficient fertilizer for cost variation of petroleum products (Naphtha), insufficient level of good quality of seed in this fast growing agriculture many companies were produce many types of seed but quality of seed was questioned, insufficient labour, lack of technology and deficit equipment. The major crises of nitrogen fertilizers demanding and pay higher amount of money farmer community fully. These fertilizers were produce soil infertility, salinity and agricultural plantation totally suffered. They were creating major crises affected to day to day life of farmer. Thus, the importance of organic sources of nutrients was recognized in current scenario in order to get higher yield without disturbing soil health [2]. In this context, integrated use of chemical and organic source of nutrients in crop production is becoming very crucial for assurance of food security on sustainable basis, which in turn not only improve the soil fertility for sustained crop productivity but also to reduce the cost of inorganic fertilizers. Different kind of organic materials such as FYM, animal manures, crop residues, composts, cow urine etc. have been used in crops but the amount and availability of nutrients in organic material vary widely, which makes interpretation of the value of nutrient supplied. Livestock wealth is deemed as the oldest wealth resource for mankind. Cow represents the Vedic values of selfless service, strength, dignity and non–violence. The "Cow" occupies the highest place of honor in Indian civilization. The five products of cow (urine, dung, ghee, milk and curd) are used in different organic systems.

Cow is the backbone of Indian culture and rural economy, and sustains our life; represent cattle wealth and bio-diversity. Cow urine is a rich source of nutrients (especially nitrogen and potassium), but usually drains out a waste material. Being organic nature, it can be used in crops without any adverse effect on environment and human health [3]. Cattle urine is a good source of nitrogen, phosphate, potassium, calcium, magnesium, chlorite and sulphate. It contains 95% water, 2.5% urea, 2.5% others (mineral salts, hormones and enzymes. Further organic nutrient spray (cow urine) can be sprayed at critical growth stage of crop to overcome the problem of the slow release nutrients of organic sources affecting crop growth.Cow urine might act as a stimulator for accumulation of nutrients in the plant biomass, proliferation of plant growth, promoting, phosphate solubilizing, a biotic stress tolerant and antagonism towards plant pathogenic fungi in the rhizosphere of plants, and enhance the total phenolic contents of the plants and controlling plant pathogenic fungi, and also it's effective in the enhancement of plant growth and soil health [4] Foliar application is credited with the advantage of quick and efficient utilization of nutrients, elimination of losses through leaching and fixation and regulating the uptake of nutrient by plants [5]. Application of cow urine has been reported to have a favorable impact, for enhancing productivity of different crops viz., mustard, maize and rice etc.

MATERIALS AND METHODS

A field experiment was carried out at Rajapolapuram village in farmers's holding, mayiladuthurai district, Tamilnadu to find out the effect of foliar spraying of cow urine based derivatives in combination with RDF on growth and yield of rice variety white ponni, as the test crop under irrigated condition during the year 2019. The experimental soil was sandy loam in texture with pH 7.25, EC 0.15 dSm^{-1}, organic carbon 3.4 g kg^{-1}. The available N, P_2O_5 and K_2O contents were 282.8, 23.4 and 267.6 respectively. The experiment was laid out in randomized block design with seven treatments. The 100 per cent recommended dose of inorganic NPK fertilizers were applied uniformly to all plots except absolute control. Different levels of cow urine based derivatives was applied based on the treatment schedule. The treatment details include, T1- NPK (RDF + water spray), T2- NPK + foliar spray of CUD @5% (SS), T3- NPK + foliar spray of CUD @10% (SS), T4- NPK + foliar spray of CUD@ 15% (SS), T5- NPK + foliar spray of CUD @5% (DS), T6- NPK + foliar spray of CUD @10% (DS), T7- NPK + foliar spray of CUD @15% (DS).The growth attributes were recorded at different growth stages of rice. The yield attributes and yield were recorded at harvest period.

RESULTS AND DISCUSSION

Foliar spray of different concentrations of cow urine derivatives as single or double spray gradually increased the growth attributes, yield attributes and yield of rice var White ponni.

Growth attributes (Table 1)

The foliar application of cowurine based derivatives along with inorganic NPK fertilizers were influenced the all growth stages of rice. The plant height was significantly increased from 69.2, 90.7 and 115.0 (cm) at tillering stage, panicle intiationsand at harvest respectively with the application of cow urine derivatives at double spray of 10%. This is on par with application of CUD at single spray of 10%. The lowest was observed in control. The application of cow urine derivatives significantly influenced the number of tillers/hill of paddy. The maximum number of tillers/hill 16.9, 19.1 and 14.9 were recorded at tillering stage, panicle initiations and at harvest stage, respectively in the treatment T_7 (CUD @ 15% DS). It is on par with application of CUD @ 10% single spray and the lowest values of 13.9, 15.5 and 12.40 were recorded at tillering stage, panicle initiations and at harvest stage, respectively were observed in control. With regard to leaf area index, they was significantly influenced by the foliar application of CUD. The highest leaf area index 3.8 and 4.7 were recorded at tillering stage and panicle initiations respectively in the foliar application of CUD @ single spray of 10% and this is on par with foliar application of CUD @ single spray of 15% (3.4 and 4.5 were recorded at tillering stage and panicle initiatios, respectively). The highest chlorophyll content 34.0 and 36.8 (SPAD) were recorded at tillering stage and panicle initiations, respectively in the foliar application of CUD @ single spray of 10% and this is on par with foliar application of CUD @ single spray of 15%. The values are 33.2 and 35.9 were recorded at tillering stage and panicle initiations, respectively. The lowest chlorophyll content was observed in control in all stages of white ponni. The lowest chlorophyll content 31.0 and 33.0 were recorded at tillering stage and panicle initiations, respectively were recorded. This suggest that foliar application of 10% SS is enough to increase the growth attributes and thereafter there was significant increase. The increased growth components with cow urine derivatives foliar application might be attributed to increased carbohydrate accumulation, cell enlargement, translocation of solute, chlorophyll synthesis and enhanced photosynthesis activity due to supply nitrogen and other major, secondary and micro nutrients from cow urine derivatives. The present findings on the significant impact of cow urine derivatives on growth parameters studied in banana and rice was confirmed by previous researchers [6,7,8,9].

Yield attributes (Table 2)

Foliar spray of different concentrations of cow urine derivatives as single or double spray caused a significant increase in yield attributes of white ponni over control at harvest stage. All the parameters increased linearly and the

maximum value was recorded with rice plants which received single spray of 10% CUD compared to other treatment combinations. The best treatment caused 21% (number of panicles m^{-2}), 62.5% (number of grains $panicle^{-1}$), 63.6% (number of filled grains $panicle^{-1}$) and 11.25% (panicle length) over control. Foliar feeding of major nutrients like N, P, K to the plants through cow urine. When nutrients required by plants are applied through foliage, there is enhancement in uptake, translocation and synthesis of photosynthetic assimilates which results into increase in various plant growth characters such as plant height, number of tillers $hill^{-1}$, leaf area and total dry matter which ultimately leads to extend in yield attributes like number of panicles $m^{-2,}$ number of grains $panicle^{-1}$ and panicle length in rice. The results are in close conformity with the findings [10,11,7,13,9] .

Grain yield and Straw yield (Table 3)

Foliar spray of different concentrations of cow urine derivatives as single or double spray caused a significant increase in grain and straw yield of white ponni over control. There was significant variation in improving the grain yield between concentrations and time of spray. Grain yield increased with concentrations and the maximum grain yield was noticed at 10 % spray at tillering stage (3917 kg ha^{-1}) and decreased at 15 % spray. Foliar spray of cow urine derivatives twice at tillering and panicle initiation did not go well in improving the grain yield except at 5% spray. The per cent increase in grain yield due to different treatments ranged from 13.4 to 108.1. The maximum straw yield was noticed with foliar spray of cow urine derivatives at 15% sprayed at tillering and panicle initiation stages (5067 kg ha^{-1}) and significantly superior rest of the treatments. The percent increase in straw yield due to different treatments ranged from 40.6 to 125 over control. Unlike grain yield, straw yield increased with time of spray. Double spray at tillering and panicle initiation stages with 5 and 15% increased the straw yield from 40.6 to 81.3 % and 62.8 to 125% respectively compare to single spray. However double spray at 10 % decreased the straw yield from 88.7 to 73.9 % compared to single spray. Grain yield depends on the synthesis and accumulation of photosynthates and their distribution among various plant parts. The synthesis, accumulation and translocation of photosynthates depends upon the efficient photosynthetic structure as well as the extent of translocation into sink (grains). The production and translocation of synthesized photosynthates depends upon mineral nutrition supplied by foliar application of cow urine derivatives. As nitrogen and potassium both are involved in protein synthesis and K helps within the translocation of photosynthates to sink, under adequate urine supply, there would have been greater translocation of photosynthates from source to sink leading thereby to production of higher number of panicle with more number of filled grains. Higher straw yield shows that at higher levels of urine application, the translocation of photosynthates to the sink (grain) was not efficient that favored more to the straw production than grain. The results are in agreement with the findings of [15, 16,17].

CONCLUSION

The study has clearly underlined the significance of usingfoliar application of cow urine based derivatives along with recommended dose of inorganic NPK fertilizers in realization of maximum rice yield and use efficiency. The study has demonstrated that foliar spray of cow urine derivatives at 10% at tillering stage with RDF recorded the maximum growth and yield of rice.

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Table 1. Effect of cow urine derivatives on plant height, LAI, tiller count and chlorophyll

Treatments	Plant Height			Number of tiller hill^{-1}			LAI		Chlorophyll content (SPAD)	
	Tillering stage	Panicle initiation	Harvest stage	Tillering stage	Panicle initiation	Harvest stage	Tillering stage	Panicle initiation	Tillering stage	Panicle initiation
T_1 – RDF + Control (Water spray.)	61.8	80.4	108.1	13.9	15.5	12.40	2.5	3.6	31.0	33.0
T_2 – RDF + foliar spray of CUD (5%) @SS	63.1	85.5	111.5	15.3	17.1	13.80	2.8	4.0	31.8	34.9
T_3 – RDF + foliar spray of CUD (10%) @SS	66.2	89.4	114.7	16.6	18.7	14.70	3.8	4.7	34.0	36.8
T_4 – RDF + foliar spray of CUD (15%) @SS	65.4	86.1	112.3	15.7	17.4	13.30	3.4	4.5	33.2	35.9
T_5 - RDF + foliar spray of CUD (5%) @DS	63.3	86.9	112.9	16.4	18.2	14.30	3.0	4.2	32.8	35.1
T_6 - RDF + foliar spray of CUD (10%) @DS	69.2	90.7	115.0	15.9	17.9	13.60	3.2	4.4	33.0	35.8
T_7 - RDF + foliar spray of CUD (15%) @DS	65.5	88.5	113.5	16.9	19.1	14.90	2.9	3.9	32.3	34.9
SE_d	1.19	1.58	2.02	0.29	0.26	0.28	0.06	0.08	0.58	0.69
CD @ 5%	2.59	3.45	4.42	0.64	0.57	0.62	0.14	0.17	1.28	1.52

SS- Single spray at TS, DS- Double spray at TS and PI

Table 2. Effect of cow urine derivatives on yield attributes of rice var. White ponni

TREATMENT	Number of panicles m^{-2}	Number of grains $panicle^{-1}$	Panicle Length (cm)	1000 grain weight (g)	Number of filled grains $panicle^{-1}$
T_1 – RDF + Control (Water spray.)	256	72	16.0	16.93	66
T_2 – RDF + foliar spray of CUD (5%)@SS	285	88	16.5	17.00	70
T_3 – RDF + foliar spray of CUD (10%)@SS	315	117	17.8	17.82	108
T_4 – RDF + foliar spray of CUD (15%)@SS	300	100	17.1	17.62	93
T_5 - RDF + foliar spray of CUD (5%)@DS	305	103	16.9	17.34	85
T_6 - RDF + foliar spray of CUD (10%)@DS	293	110	16. 6	17.08	100
T_7 - RDF + foliar spray of CUD (15%)@DS	287	92	16.4	17.40	90
SE_d	4.58	1.55	0.19	0.39	2.00
CD @ 5%	9.98	3.39	0.42	NS	4.36

SS- Single spray at TS, DS- Double spray at TS and PI

Table 3. Effect of cow urine based derivatives on riceyield var.whiteponni

Treatment	Grain yield ($kgha^{-1}$)	Per cent increase over control	Straw yield ($kgha^{-1}$)	Per cent increase over control
T_1 – RDF + Control (Water spray.)	1883	-	2252	-
T_2 – RDF + foliar spray of CUD (5%)@SS	2135	13.4	3167	40.6
T_3 – RDF + foliar spray of CUD (10%)@SS	3917	108.1	4250	88.7
T_4 – RDF + foliar spray of CUD (15%)@SS	2600	38.1	3667	62.8
T_5 - RDF + foliar spray of CUD (5%)@DS	2917	54.9	4083	81.3
T_6 - RDF + foliar spray of CUD (10%)@DS	2250	19.5	3917	73.9
T_7 - RDF + foliar spray of CUD (15%)@DS	2533	34.5	5067	125
SE_d	177		173.	
CD @ 5%	386		378	

SS- Single spray at TS, DS- Double spray at TS and PI

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REVIEW ARTICLE

A New Era in Management of Diabetic Neuropathy

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ABSTRACT

Diabetes Mellitus or hyperglycemia is the most widespread and critical disease as its onset and longer duration gives rise to number of other complications, disease & disorders. Long run of hyperglycemia is followed by micro vascular complications like retinopathy, nephropathy, neuropathy & macro vascular complications like cardiovascular diseases, atherosclerosis, stroke & peripheral arterial diseases. Diabetic neuropathy is a painful disorder which affect both peripheral and autonomic nervous system accompanied with complex pathophysiology that involves increase in the activity of polyol pathway, hexosamine pathway, advanced glycation end products and activity of protein kinase C (PKC) which directly or indirectly enhances oxidative stress that leads to diabetes induced nerve injury, neuronal degeneration or death of nerve cells. Specific inhibitors or anti's of these pathways have not shown much success due to the side effects that outnumber their therapeutic effects but in order to control the pain, tingling effect, numbness or loss of sensation pharmacotherapies & non pharmacological therapies are used for symptomatic treatment. Cell therapy, protein and gene transfer, production of antibodies, new diagnostic techniques are also used for the treatment of neuropathic pain.

Keywords: Neuropathy, Polyol pathway, Hexosmine pathway, Pharmacotherapies, Non pharmacological therapies.

INTRODUCTION

When there is an inappropriate production or disturbed functioning of endocrine system it causes hormonal imbalance which leads to a medical condition probably referred to as endocrine disorder. Diabetes mellitus is also an endocrine disorder that either resists the insulin secretion due to factors like auto immune responses, genetic & environmental factors (Type I) or destruct β-cells (TYPE II) [1, 2] .Elevated blood glucose level (hyperglycemia) makes it way forward to give rise to acute or chronic complication affecting the large and small blood vessels. On the basis of type of vessels being damaged, the complications due to hyperglycemia are grouped as microvascular and macrovascular complications. Microvascular complication includes retinopathy, neuropathy and nephropathy whereas macrovascular complications include cardiovascular diseases and stroke[3]. Diabetic neuropathy is the most common complication of both Type I and Type II Diabetes Mellitus affecting majority of population all over the world.50% of population is affected with diabetic neuropathy most of the time it remains asymptomatic. Diabetic Neuropathy is defined as the composite disorder which is characterized by progressive loss of sensation mainly due to the disparity between nerve fibre damage and repair in diabetic patients excluding other possible factors[4, 5] . The loss of functioning of nerves or diabetes induced impairment in nerve depends on the type of nerve being affected. It may be the sensory (afferent) or motor (efferent) nerves or both, signs and symptoms varies with the type of nerve damaged[6, 7].

Clinical signs and symptoms[8, 9, 10]

- Intense stabbing pain, balance problem, paralysis, cramping
- Depression, anxiety, sleep disorder
- Tingling, allodynia, electrical sensations
- Numbness, loss of sensation,
- Slowing of sensory nerve conduction velocity (SNCV) & Motor nerve conduction velocity (MNCV)
- Foot infection, ulcers & charcot's joints
- Burning, tactile hyperesthesia, dysesthesia

Classification of Diabetic neuropathies [11, 12]

The diabetic neuropathy may be classified on the basis of type of nerve involve whether it is sensory or autonomic or motor nerve, position of injury of nerve that is focal, multifocal or generalized and time course of disease either its chronic or acute.

A. Diffuse neuropathy

1. Distal Symmetric Sensorimotor Polyneuropathy (DSPN)
   - Large fiber neuropathy
   - Small fiber neuropathy
   - Large and small fiber neuropathy (Mixed)
2. Autonomic Neuropathy
   - Gastrointestinal neuropathy
   - Cardiovascular neuropathy
   - Urogenital neuropathy
   - Sudomotor neuropathy
   - Vasomotor neuropathy
   - Symmetric Proximal Limb Motor Neuropathy (Amyotrophy)

B. Focal Neuropathy

- Cranial Neuropathy
- Radiculopathy / Plexopathy
- Entrapment Neuropathy
- Asymmetric Lower Limb Motor Neuropathy (Amyotrophy)

Pathophysiology

There is no such credible hypothesis to mark the actual cause or reason for diabetic neuropathy. There is no explanation regarding why some patients experience pain and while others not. Oddly severity of neuropathy is not anyhow linked with the presence or absence of nerve injury. Hyperglycemia is one of the major factors that develop complications. Many other such elements that upsurge neuropathic pain are hyperactivity of polyol pathway, hexoamine pathway,AGE pathway,Protein kinase C pathway, Nitrosative and oxidative stress, changes in microvessels, activation of microglial cells, brain plasticity, dyslipidemia, inflammation, growth factors, impaired insulin signaling, impaired endothelial function, apoptosis and Poly ADP-ribose polymerase pathyway [13, 14].

1. **Polyol pathway**: In this pathway there is an increase in aldose reductase activities which hike up the sorbitol & fructose level leading to excess accumulation and decreases free nerve myoinositol bringing up variance in nicotinamide adenine dinucleotide phosphate which results in reduction of glutathione prevailing **oxidative stress**[15].

2. **Advanced Glycation End Products (AGEs):** Non-enzymatically glycated proteins like haemoglobin, plasma albumin, lipoproteins, collagen & fibrin.
   AGE-Receptor of AGE (RAGE) interactions → Activation of transcription factor nuclear factor- κβ (TNF- κβ)

INFLAMMATION AND APOPTOSIS IN NEURONAL CELLS [16]

3. **Protein Kinase C activation:** Increase in Diacylglycerol Production

Increase PKC activation results in alteration in vascular permeability (vasoconstriction)
Reduced Blood Flow

NERVE DEGENERATION[17]

4. **Hexosamine pathway** : Over expression of TGF-β1 (Transforming Growth Factor- β1) & PAI-1 (Plasminogen activator inhibitor-1) [FIG

INFLAMMATION → NERVE DYSFUNCTION OR NERVE CELL DEATH[18]

DIAGNOSIS OF DIABETIC NEUROPATHY

Hyperglycemia is the utmost reason to stress out Schwann cells and making changes either in vessels or basement of the nerve fibre putting in with neuronal and microvascular damage. Patients with diabetes often suffer with pain and many are out there without any symptom of pain like stimulus referred to as asymptomatic. Those with no symptoms are presented with foot ulcer as the first clinical symptom but other can follow up with symptoms like numbness, tingling sensation, loss of sensation, etc. Some of the diagnostic methods are mentioned below[19].

Neurometer

It is a Quantitative Sensory Testing (QST) neurodiagnostic non-invasive device that measures the sensory nerve function the current perception threshold (CST). It detects the functionality of nerve fibres at a frequency of 2000Hz for Aβ fibers, 250Hz for Aδ fibers and 5Hz for unmyelinated C fibers. It can quickly and painlessly pick up hyperaesthesia and hypoaesthesia. Most sensitive device than vibration perception threshold (VPT) and monofilament testing[20].

DPN-Check

It's an accurate, fast, cheap, user friendly, handy device which is used to measure the nerve conduction velocity without the need of neuroelectrophysicist. It only takes 3 minutes to test NCV as compared to Nerve Conduction Studies (NCS) which is time challenging. It can also detect early DPN. Neurometrix Inc., Waltham, MA are the DPN check available in market[21].

Sudomotor Testing

Nerves that stimulates sweat glands are known as sudomotor nerves. In diabetic neuropathy these nerves are dysfunctional and lead to disturbed function of sweat glands which form foot ulcers. Methods to determine the functioning of sudomotors are:

- Quantitative Sudomotor Axon Reflex Test (QSART)
- Thermoregulatory Sweat Test
- Quantitative direct and indirect reflex test
- Neuropad
- Sudoscan[22,23, 24].

Corneal and Retinal Innervations

Eyes are the extension of Central Nervous Systems (CNS) which is thickly supplied with sensory nerve fibers and few autonomic fibers. Diabetic neuropathy damages the nerve fiber supplying the cornea reaching to a condition called retinopathy[25].Corneal Confocal Microscopy (CCM) is a technique with sensitivity percentile up to 68-92% and specificity ranging up to 40-64% which deals with retinal nerve fiber lengthP26]. Optical Coherence Tomography (OCT) is another technique used to recognize the retinal fibers lost in neurological disorders[27].

Skin Biopsy and Quantification of Intra-epidermal Nerve Fiber Density (IENFD)

It is a test which is used to detect the damage occurred in small fibers. This is measured using Punch Skin Biopsy a gold standard test that detects the neuropathy in small fibers. Small epidermal unmyelinated sensory fibers through skin biopsy evaluate intra epidermal nerve fibers. IENFD in correspondence with other neuronal role have 61–90% of sensitivity and specificity of 64–82.8% to determine DPN. An easy and quick way to diagnose DPN but it is must to have the laboratory equipment and skill to examine[28].

Microneurography

It is a neurophysiological method which uses metal electrode to inspect the neural traffic in both myelinated and non-myelinated nerves. Microneurography has successfully target peripheral nerves and reduced neuropahic pain. Since it is time consuming and invasive method only few studies had engaged it in diabetic subjects[29].

Nerve Biopsy

Nerve biopsy is a method which is used to determine the reason behind the symptoms like numbness, tingling sensation, prickling pain, weakness, tremors, cramps, balance issue, etc. It basically requires a small piece of nerve taken out via incision and observation under microscope to detect the cause of the symptoms mentioned above which are either due to degeneration of nerve, inflammation of nerves, injury or any other condition. It is also used for assessing the therapeutic effect of drugs in potentiating remyelination of nerve fibers and regeneration of axon [30].

TREATMENT FOR DIABETIC NEUROPATHIC PAIN

Depending upon the intensity and duration of pain patients are advised to take medication in lowest dosage regimen. Poly pharmacy is probably avoided but looking on to the severity of symptoms it is provided though there are data that supports the use of multidrug therapy. Drugs with maximum possible side effects are discontinued; those with minor aftereffect are still being used[31]. As for the treatment and management of diabetic neuropathy it is a pathogenic mechanism, alteration in fit fine metabolic cycles creates irregularities in normal body functioning and interrupt the ease of process. In diabetes elevated blood glucose level disrupts the insulin secretion,

hyperactivation of many pathways which hop onto the painful symptoms and muscle weakness. Glycemic control, treating the symptoms and sequencing the pathogenesis are methods used to treat pain in diabetic neuropathy[32].

Glycemic control

Either due to β-cells destruction (T1DM) or insulin resistance (T2DM) there is absence or decrease in insulin production which leads to hyperglycemia; Hyperglycemia through various pathways and other factors it leads to neuropathic pain. Antihyperglycemic agents are used to overcome the elevated blood glucose level. Commonly used oral agents are:

i. Sulfonylureas (Tolbutamide, Glibenclamide): Inhibits potassium sensitive ATP channels **(K_{ATP})** and increases insulin secretion.
ii. Biguanides (Metformin): Activation of **Adenosine monophosphate dependant protein kinase (AMPK)** Suppress hepatic gluconeogenesis & enhance glucose uptake**.**
iii. Thiazolidinediones (Pioglitazone): **Peroxisome proliferators-activated receptor γ (PPAR-γ)** activation Stimulate GLUT 4 & brings down insulin resistance.
iv. α-Glucosidase inhibitor (Acarbose, Voglibose): **Inhibits glucosidase** and decreases digestion & absorption of glucose.
v. Dipeptidyl peptidase-4 inhibitor (DPP-4) (Sitagliptin, Vildagliptin): Stimulate **Glucagon like peptide (GLP-1) & Gastric inhibitory peptide (GIP)** Lowers hepatic glucose production & intensifies insulin secretion[33, 34] .

Pathogenetic and symptomatic treatment

Below is the list of drugs used in both mechanisms and symptoms based therapy along with their doses, side effects and mechanism involved.

Drugs Class	**Mechanism**	**Dose**	**Side Effects**
Epalrestat	Aldose reductase inhibitor. **Delay sorbitol accumulation** in sciatic nerve/other tissues. Protein kinase C inhibition. Increases **endothelial nitric oxide** production**.**	50mg three times/day or 150mg/day	Abdominal pain, diarrhea, headache, dizziness, vomiting[35].
Benfotiamine	PKC & Hexoamine pathway inhibitor. **Decreases diacylglycerol** production. Decreases AGEs & inhibits hexosamine pathway. Improves Nerve Conduction Velocity (NCV), pain and reduces inflammation.	150-300 mg twice daily	Alopecia, body odor, hypotension, weight gain, upset stomach, nausea[36].
Ruboxiastaurin (RBX)	PKC inhibitor. Binds to active state of **PKC-β**. Disturbs ATP binding & Inhibits phosphorylation of substrate. Increase blood flow.	32 mg/day	Elevation of hepatic enzymes, vomiting, diarrhea[37].
N-phenacylthiazolium	AGEs inhibitor. Inhibition or reverse of AGEs via	-	-

bromide (PTB) γ-linolenic acid	**cross link breakers** between **collagen and albumin [38].** Prevents neuron degeneration Improves **NCV**. Anti-inflammatory action [39].	-	-
Amitryptyline & Nortriptyline	Blocks pain signals via inhibiting uptake of biogenic amine **Noradrenaline (NA) & 5-Hydroxy tryptamine (5-HT**) also known as **serotonin**.	10 to 150 mg/day 25 to 150 mg/day	Severe rash, excessive sweating, frequent urination, change in appetite, weakness, Decreased sexual ability[40].
Gabapentin & Pregabalin Valporate	Binds to **L-type voltage gated sodium channel** (α2δ site), decreases Calcium**.** Blockade of **voltage gated sodium channels** & increase in GABA	2400-3600mg/day 300-600 mg/day 1000-1200mg/day	Peripheral edema, tremors. Thrombocytopenia, somnolence[41].
Fluoxetine & Paroxetine	Selective inhibition of reuptake of **serotonin** excluding **norepinephrine** Increases **Serotonin** activity**.**	40mg/day	Heartburn, anxiety, yawning, confusion stomachache, weight loss, decreased appetite, sleepiness.

Topical medications:

1. Lidocaine (5%): Neuronal membrane stabilization through **voltage gated sodium channels antagonisation**; decreases neuronal discharge.
2. Capsaicin (8%): Reduction of **substance P** & alteration in other pain involved mechanism.
3. Clonidine (0.1%): Binds to ***α*-2 adrenoreceptor** ; prevents transmission of pain signal.
4. Isosorbide Dinitrite Spray: Prevents pain via **vascular smooth muscles relaxation**.
5. Intradermal botulinum toxin type A (BTX-A): Works by **blocking the neuronal release**; reduces peripheral sensation[44].

Drugs with severe side effects are withdrawn from the market whereas others are under clinical trials. Some of them are:

1. Ranirestat (Phase II clinical trial): Improves **Nerve conduction velocity (NCV)** [Dose 20mg/day] [45].
2. Sorbinil: Prevents neuronal dysfunction & reduces sorbitol accumulation.
   Adverse effect: Hypersensitivity reactions.
3. Tolrestat: Inhibits conversion of glucose to sorbitol.
   Adverse effects: Splenomegaly, Respiratory distress syndrome, Increase in liver enzymes.
4. Ponalrestat: Act by inhibiting conversion of glucose to sorbitol.
   Adverse effect: Fatal hepatic necrosis [46].
5. Aminoguanide: Prevents formation of AGEs by reacting with 3-deoxyglucosone (precursor of AGEs) by trapping reactive carbonyl.

Adverse effect: Withdrawn from clinical trials due to toxicity[47].

NOVEL INTERVENTIONS FOR THE TREATMENT OF NEUROPATHIC PAIN

Anticonvulsant, NSAIDs, opioids, SSRIs, SNRIs, ARIs, PKC inhibitors and many more drugs are still used for treating the neuropatic pain. Neuropathic pain affects majority of population, it's not only specifically related

diabetic complication. There are some novel technologies and drug formation which are being used or are still under clinical trials.

THERAPIES	**TARGET**	**MECHANISM**	**REFERENCES**
Gene Therapy [SB-509]	Vascular Endothelial Growth Factor (VEGF)	Activates VEGF-A, restores, preserves & rejuvenates peripheral nerves.	48
Humanized Monoclonal Antibody Tanezumab(Phase I & II)	IgG2 antibody	Disrupts Nerve Growth Factor (NGF) & its receptors interaction.	49
Micro-RNAs[miR-146a] **Cell Therapy** Bone Marrow Mononuclear Cells [BM-MNCs]	Plasma & sciatic nerve tissue Angiogenic & Neurotrophic factors	Suppress pro-inflammatory genes. Neovascularization	49 50
Mesenchymal Stem Cells (MSC2)	Inflamed tissues	Decreases pro-inflammatory cytokines.	51
Growth Factors VM202 (Plasmid containing HGF)	Hepatocyte Growth Factor (HGF)	Inhibits spinal cord glial activation & factors that produces pain in dorsal root ganglion neurons.	49
Islet Neogenesis Associated Protein (INGAP) **Angiotensin-II receptor (AT_2R) antagonist** (EM4401)	NGF AT_2R	Stimulates NGF Inhibits hyperexcitability & rapid firing of sensory neurons.	49 52
Nav Channel Antagonist Vixotrigine (Phase II) VX-150 (Phase II) Gibinetide (ARA290)	Sodium channel Nav 1.7 channel Nav 1.8 channel Innate repair receptor	Ion channel modulation using definite genes. Activates anti-inflammatory pathways.	53
P2X3 antagonist Gefapinant [MK-7264 or AF-219] (Phase II/III) BLU-5937 (Phase I/II)	Purinergic P2X3 receptor	Inhibits Purinergic P2X3 receptor in PNS.	53
Dual inhibitor of enkephalinases (DENKI) PL37	Enkephalin degrading enzymes	Inhibits enkephalinase enzyme & stimulates opioidergic pathway	53

Other therapies include:

1.**Neuromodulation:** An implantable and non-implantable technology which modulates the electrical and chemical signal in nervous system in order to alleviate the pain. It involves;

a. Intrathecal pain therapy (IT): Approved for treating refracting pain.
Drugs used are morphine (0.1mg-0.5mg/day) & ziconotide (1.2mcg/day)[54].

b. Spinal cord stimulation (SCS): Spinal cord stimulator a device that reduces the pain by sending weak electrical signals to the spinal cord. Further includes:

i. Tonic SCS: Using a frequency of 40-100Hz it generates electrical parathesia which overruns the pain in affected area[55].
ii. Sub-parathesia SCS: SCS at high frequency (10000Hz) & burst stimulation along with medical therapies overlaps the pain proving with remarkable pain relief[56].
iii. Dorsal root ganglion stimulation: Provides way better pain relief than Tonic SCS & Sub-parathesia SCS. A neurostimulation therapy which is deviced to treat untreatable pain of lower extremities like foot, hip, knee or groin[57].

2. **Trancutaneous electrical nerve stimulation:** Neuromodulation device that generates mild electrical current when placed on painful area in the form of patches using the electrical signals for pain relief[58].
3. **Acupuncture:** A pseudoscience which involves use of needles to prick the specific points in body which will tend to make biochemical changes by releasing chemicals & stimulates central nervous system for effective treatment[59].
4. **Photobiomodulation:** Also known as Low-level laser therapy. This therapy helps to improve healing of tissue by using infra red light[60].

CONCLUSION

Diabetes Mellitus or hyperglycemia is a disease that can't be cured completely but can be controlled. Since, it is the key to Diabetic neuropathy; it is must to have glycemic control. Use of insulin for Type 1 diabetes and oral anti-hyperglycemic agents for Type 2 diabetes are the methods used to control increased blood glucose level.Therapeutic agents acting directly on different pathways responsible for neuropathic pain, has shown limited success but in a matter of time using the detailed novel information and new targets we'll be able to achieve our goal to prevent neuropathy in diabetic patients.Though mechanism based medication failed in its beneficial effects, still there are various drugs which can at least be used to make patient free from pain for a time being. These includes different classes of drugs like SSRIs, SNRIs, TCAs, Anti-epileptics & opioids which are used to treat painful symptoms of neuropathy along with way more side effects. Despite having numerous options; worrisome thing is that these drugs are accompanied with side effects sometimes adverse effects which make these agents less reliable. Nutritional supplements like zinc, magnesium, vitamin D, vitamin B, combinations of drugs, new therapies like Intravenous therapies (Lidocaine, Ketamine), nanotechnology developments, antagonists for different pathways responsible for vascular changes, change in diet, lifestyle, exercise, accepting natural therapies are few ways to overcome the diabetic complications. Development of nano particles, recently developed therapies, discoveries of novel drugs, finding of new pathways to prevent the disease, identifying & understanding the interconnections between different pathways, the reason behind the problem, factors involved are the conceptual layout of any disorder or disease. There is no complete understanding behind the reason of diabetic neuropathy. More relevant facts, studies, researches are required. Discoveries of new targets & agents soon will provide us with new hope.

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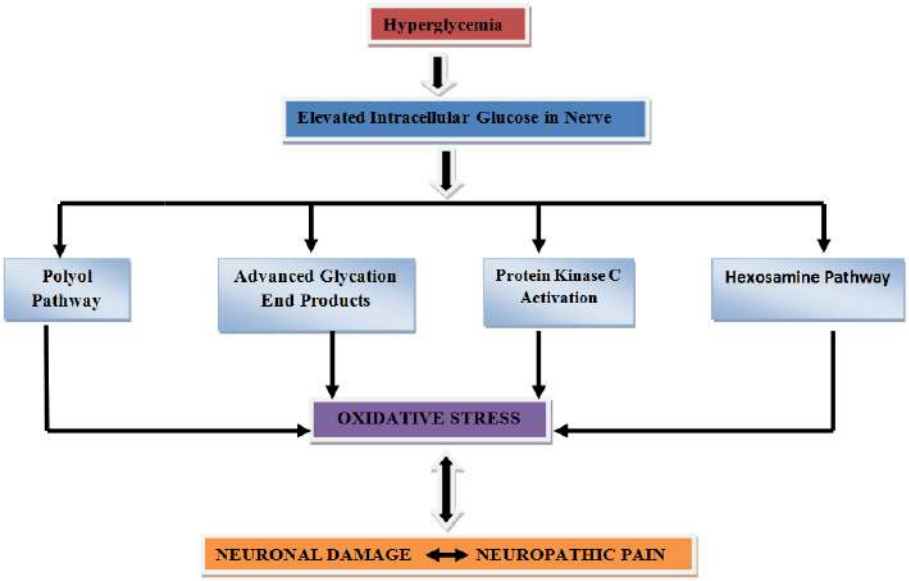

Figure 1. Pathophysiology of diabetic neuropathy

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REVIEW ARTICLE

Biosimilars in India-Current Status and Future Perspectives: A Review

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ABSTRACT

Biosimilars are defined as officially approved remake of innovator bio-therapeutic products that patent has expired. Biosimilars the 'generic' versions of biopharmaceuticals, still enter pharmaceutical market, to treat a spread of disease. In India various biosimilars are available like monoclonal antibodies for treating various malignant and immunological disorders, growth factors like erythropoietin and granulocyte colony stimulating factor (G-CSF), Human insulin for treating diabetes etc. In recent few years, there are several epic biological products are going of patent which has generated Associate in Nursing abridged route for the biosimilars products that depends on the in -depth equivalence testing against Reference Biological Product (RBP reassuring product`s quality, safety and effectualness. The primary draft guideline for biosimilars was established by Europe, subsequently Japan and USA as developed the draft guidelines. While recently India has established the biosimilar guideline in June 2012 for the pre -and post-marketing approval of comparable biologics. India has vigorous pharmaceutical industry for the drug while it can become an emerging marketplace for biopharmaceutical drug. India is one amongst the leading manufacturers of similar biologics. The articles say about the present status, Indian Biosimilar market, current and future state of the Indian biologics market and future perspectives.

Keywords: Biosimilars, reference biological product, similar biologics, biopharmaceutical drug, Indian pharmaceutical market.

INTRODUCTION

Biopharmaceutical drugs became a necessary part modern pharmacotherapy. These comprise proteins derived from recombinant DNA technology and hybridoma techniques. The pharmaceuticals market in India is incredibly

exclusive and has demonstrated very high potential within the last number of decades. The world has ranked tenth globally in terms of valuable and ranked third in terms of volumes. The Indian pharmaceutical market has the potential to succeed in USD 70 billion in future growth scenario. India's ranking is among the 12 top biotech destinations within the world with the third position within the Asia pacific region. India's biotechnology segment one in every of the fastest-growing sectors with the turnover of USD 7bn during the year 2015, and since then it`s has been growing at the speed of sixty-three % annually (India pharma, McKinsey & Company report, 2020).Due the rise in patent expiries for biologic drugs, there exists a valuable opportunity for the event of more productive biopharmaceutical industry in India. In keeping with FDA definition, biosimilars are licensed by FDA as they`re clone of to the approved reference product, and shown to own no clinical difference from the biologic reference product. In 2006 EU Common Market has approved the primary biosimilars and now the biosimilars drugs reaches quite 700 in numbers. The biosimilars guidelines of India are in regulations with the EMA and WHO. India pharmaceutical companies are enhancing their manufacturing skills, and for clinical trials, they`re working along with pharmaceutical companies worldwide.

Regulatory Bodies for Approval of Biosimilars

In India the regulatory bodies to blame for approval of 'similar biologics` under the guidance of the Department of Biotechnology (DBT-under the Ministry of Science and Technology) through its review committee on genetic manipulation(RGCM), and also the Central Drug Standard Control Organization (CDSCO -under the Ministry of Health and Family Welfare). The govt of India is now providing necessary infrastructure to support industry for funding and global collaboration to bridge the technical knowledge gap. For instance, the govt of India has also collaborated with the numerous foreign universities to bridge the knowledge and technology gap. Bio-pharma mission which an industry academia collaborative mission and is implemented by Biotechnology Industry Research Assistance by the Department of Biotechnology and United Nations commission. Draft regulatory guidelines for `similar biologics` was announced and released by the India, at the BIO industry conference in Boston, USA, on 19June 2012. Finalized pointers were then revised and updated, with new pointers, that came into impact in August 2016.

Indian Biosimilar Market

Biosimilar product should have resemblance with the reference product in terms of quality, stability, characterization, specification, efficacy, safety, diagnosing attributes, clinical attributes, pharmacokinetics and pharmacodynamics, toxicity and immunogenic studies (study on the Indian pharmaceutical industry, 2015) India encompasses a huge share of the biosimilar market, and that they are expected to become a progressively vital a part of the pharmaceutical ecosystem (Ray Tanmoy,2017). Within the domestic market, there are above 20 biopharmaceutical companies actively functioning on biosimilars development. Till date, quite seventy biosimilars merchandise are been approved in Bharat and these figures are unendingly increasing. Among these, quite 50 biopharmaceutical products are permitted for marketing in India which incorporates insulins interferons, development hormones, filgrastim, proteins and streptokinase. And with more 60 biosimilars within the development pipeline, the industry is certain to establish itself in therapeutic areas like cancer treatment, immunological disorders and diabetes. And biosimilars makers are specifically inquisitive about leading biologics like Avastin, Humira and Levemir with recent patent expiry. Now, the Indian manufacturers are directing their concentration on more biosimilar production as many follow-on biologics are going off patent within the coming years. And it`s anticipated that there`ll be an increase within the market share of follow on biologics within the global biopharmaceutical market (study on the Indian pharmaceutical industry, 2015). Presently, India is booming as a significant contributor within the world biosimilar industry. One in every of the numerous strength India has is that it`s the foremost significant number of USFDA approved manufacturing plants outside the US.

Current Status

India contains a thriving biosimilar ecosystem compared to other countries and since of that Indian Pharmaceutical companies have risen because the global market leaders in biosimilar, India approved its first biosimilars much before the US and Europe. The primary biosimilars was approved and marketed in India in 2000 for viral hepatitis,

although no specific guidelines available at that point for the event and marketing of biosimilars in India. India is now become a largest exporter of generics within the world, accounting for 25% of the US generic market share in 2018 in step with the Pharmaceutical Export Promotion council of India. Indian biosimilar market was approximately USD dollar 250 million and growing at a compound annual rate of 14%.

Export of Indian Biosimilar Product

By 2030, India become sixth-largest marketplace for pharmaceuticals, and it`s firmly established itself within the global biopharmaceutical market. Many of the Indian Pharmaceutical companies are preparing to step into the world biosimilar market. As per the report of Associated Chambers of Commerce of India`s 2017, biosimilars represents a 30% compound annual growth report. They`re worth$2.2 bn out of $32bn total Indian Pharma market and are estimated to achieve $40bn by the year 2030. The expiry of a spread of biologic patents In upcoming years will further aid this growth. In India, there`s an energetic pipeline of biologics segment within the list of the highest Pharmaceuticals firms named as Intas Biologicals, Biocon, Dr Reddy`s Laboratories, Zydus Cadila, Reliance life Sciences, Lupin Pharma, Wockhardt etc (Indian biosimilar market to be worth $40bn by 2030,2016). "Indian biosimilars industry" was near to $300 million in 2015 was reported by 'ASSOCHAM-SATHGURU' in Economic Times. However, with the introduction of a replacement regulatory policy in India and increased affordability that biosimilars offer, the domestic market will expected to grow at an accelerated pace and reach the target of $40billion by 2030 and can command 20% share in global market. India tops the chart for approved biosimilars and clinical trials surpassing USA and Europe :10th Annual Pharma IPR India Conference in Sep-24.

Biosimilar Approvals and Launches In 2021

Almost like last year,2021 saw relatively few new biosimilar approval from FDA, with only four newly approved biosimilars: Mylan's (no Viatris's Semglee and Eli Lilly's Rezvoglar" the primary and second Lantus (insulin glargine) biosimilars; Samsung bioepis's Byooviz™, the primary Lucentis (ranibizumab) biosimilar; and Coherus's Yusimry™, a Humira (adalimumab) biosimilar. The continued slow pace of approvals in potentially the results of the COVID-19 pandemic and related delays in clinical trials and facility inspections by FDA, yet as re-prioritization and shifting of agency resources towards COVID-19 vaccines and coverings. Regulatory agencies in 2021 focused on three interrelated issues: (1) Decreasing anti-competitive behavior within the biosimilar space, (2) lowering biologic drug prices, and (3) the providing further guidance to clarify the regulatory pathway for biosimilars

Future State of the Indian Biologics Market and its Current Status

The World biosimilars market is predicted to succeed in $35.7billion by 2025, up from $11.8% billion in 2020, at a CAGR of 24.7% Thanks to the Association of Biotechnology Led Enterprises, India`s biologics market will grow at a compound annual rate (CAGR) of 22% to that $12billion by 2025. The market currently is dominated by simple biologic, like therapies for the treatment of diabetes (insulin)oncology (EPO and mAbs) autoimmune, and cardiovascular diseases. Similar biologics for insulin and EPO enjoy 80 to 85% market share in India. However, complicated biologics including mAbs in Asia Pacific is predicted to be $5.94bn in 2020 and his anticipated to achieve $10.9bn through 2025 at CAGR of 4.52%, therein panorama India is that the fastest growing country.

The Opportunities & Challenges of India's Biologics Market

In latest years, the govt has demonstrated strong initiative to push the pharmaceutical sector, along with a $1.3bn fund to inspire organizations to fabricate pharmaceutical substances locally through 2023. The National Health Protection Scheme is that the largest government funded Health care program within the world, and it`s expected to benefits 100 million poor families within the country by providing up to $7,732.20 per family annually. The Indian Pharmaceuticals market is exclusive in many ways - 70% of the retail market, and native players have enjoyed a dominant position driven by formulations development capabilities and early management. While the Indian Pharmaceutical market ranks 10 globally in terms important, it`s ranked third in volume of pharmaceutical products. McKinsey estimates that the Indian Pharmaceutical market was valued at $ 12.6 billion in 2009 and expected to succeed in US$ 100 billion by 2025 for Indian Brand Equity.

Future Perspective

The Expansion of worldwide biopharmaceutical market is influenced by various factor like desired results of clinical trials, emerging pressure to diminish healthcare expenditure increase within the incidence rate of chronic disease, cancer etc. The proportion of the biopharmaceutical zone is forecasted to travel through growth each with inside the Indian and international pharmaceutical marketplace. Within the upcoming decade, there would be an upsurge within the number of existing biologics drugs going off -patent, which results in an increase in demand for biosimilar drugs. However, sure disputes including production complexities, novel techniques through biologics drug manufactures, costs, stringent regulatory necessities in evolved and growing nations will preclude the access of recent gamers and limitation the advance of this marketplace. By searching on the developments with inside the most constructive situation, its miles anticipated that in during India through the year 2030 the biosimilars pharmaceutical marketplace global might be of $240 bn and therefore the home marketplace attain may be around $40 billion. Institute of Medical Sciences healthcare report also envisages the same opportunity for Indian biopharmaceutical companies related to manufacturing and marketing of biosimilars. Therefore, the Indian Biopharmaceutical firms can attain specialization in the biosimilar sector and further propagate it to established markets. Thus, making India a main contributor within the direction of this section and this necessitates the existence of a specified and streamlined procedure so the Indian manufactured products is at par with the globally regularly occurring standards, and more export opportunities will be harnessed. These measures will safely deliver India with ammunition to compete with other developed countries in terms of regulatory aspects and export of biosimilars. Thus, it`s essential to foster the colorful industry landscape and support the biosimilar pharmaceutical industry in real value realization. It`s pertinent to effect important facts and proper stock to all or any participants. So, they will be prepared to contest in commercial encounters both in domestic in addition as international markets.

CONCLUSION

Biosimilars have the capacity to reinforce patient access to high level drug therapies in addition as alleviates the financial strain that chronic illness place upon healthcare systems worldwide. India has firmly established itself as a worldwide player as a marker of comparable biologics due to its burgeoning population. Although, the capacity is excessive and expectation is large for India, the disputes are good sized and overwhelming hold the leadership. To attain truth potential and continues have to upgrade their technology and must improve the manpower skills. For this, they require a permitting, surroundings from the authorities and regulatory agencies. Thus, India will attain its international chief situation in 2030.

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Table No: 1 'Similar Biologics'approved and marketed in State

Product Name*	Active Substance	Therapeutic area*	Approval/ Launch date in India	Company
AbcixiRel	abciximab	Angina,Cardiac ischemia	23 Apr 2013	Reliance life Science
Actorise	Darbepoetinalfa	Anaemia, Cancer, Chronic failure	6 Jan2014	Cipla
Adfrar	adalimumah	Ankylosing spondylitis, RA	11 Jan 2016	Torrent pharmaceuticals
Bevacirel	bevacizumab	Colorectal cancer	10 Jan 2016	Reliance life science (Lupin)
Bivoc-B	Hepatitis vaccine	Hepatitis B	2000	Wockhardt
Cizumab	bevacizumab	Colorectal cancer	27 Jun 2016	Hetero
Cresp	darbepotinalfa	Anaemia, cancer, CRF	23 Mar 2010	Dr.Reddy's Laboraties
Darbatitor	darbepoetinalfa	Anaemia, cancer	2014	Torrent Pharmaceuticals

RESEARCH ARTICLE

Karl Pearson's Correlation Coefficient for the Physico-Chemical Parameters of Ayyanakere Lake water of Chikmagalur District, Karnataka

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ABSTRACT

The study of the physico-chemical water parameters of Ayyanakerelake was conducted from Chikmagalur district of Karnataka, India. The datas obtained were subjected to Karl Pearson correlation coefficient matrix. Correlation coefficients revealed positive and significant correlations between the pairs of parameters inlake surface water. This is extremely critical method of examination as it address how increments in one of the quality or physico-chemical boundary of water prompts increment or reduction in other parameters experimentally with correlation regression equation. The significant varieties are identified with anthropogenic exercises (water system rural, development exercises, getting free from land). The current investigation, notwithstanding, is accepted to fill in as a pattern information for additional examinations. Future exploration should subsequently vital commitments to the current information on the spatial varieties of surface water quality and focus on the examination of transient varieties of water quality in the lake. In future, the lake is bit by bit tending towards eutrophication.

Keywords: Water Analysis, Oligotrophic water body, Karl Pearson Correlation Coefficient, Ayyanakere Lake.

INTRODUCTION

Water is appropriately depicted as the actual premise of life, the leftover being put away for significant stretches in the seas, ice covers and springs. Seawater contains about 97% of the absolute accessible water on the earth; staying 3% include all freshwater sources. Expanding requests on water supplies have influenced both surface and sub-surface water supply to enormous degree and numerous spaces of the nation are plainly giving obvious indications of intense water emergencies. Some obvious impacts incorporate little streams becoming occasional, unconfined springs giving indications of water table decrease and more profound springs showing diminished and dubious yields. This load of impacts, brought about the clients depending on drastic actions like sinking wells and penetrating bore wells and tube wells to discover falling water levels and expand questionable water supplies along these lines. The issue acquires an inside and out various measurement in spaces of low or potentially whimsical precipitation, a typical element in numerous areas of India. The lentic water bodies serve to check fast water streams in the valleys and forestalls the seriousness of dry spells during dry months. They assume a critical part in ground water re-energizes and goes about as channel for particular sorts of squanders and dissolvable toxins. They give reusing zone to crude materials and rotting natural issue. They have tasteful worth separated from giving haven to many birds and other significant aquatic life. They additionally fill in as entertainment assets. An immediate technique for the assessment of the possibility of an aquatic biotope is the assessment of the pace of its essential creation, where it starts the essential obsession of energy and its ensuing exchange to higher trophic levels (Wetzel, 2001). New water bodies are a significant piece of the normal scene and critical for human development and biodiversity. The escalated land use changes and the serious utilization of freshwater water bodies for an assortment of purposes has lead to huge corruption of the freshwater water bodies all around the world during the last century. Freshwater accessible to human, animal and plants isn't equitably disseminated on the earths surface.

Sharp contrasts exist in the measure of all out yearly precipitation in various pieces of the world and furthermore change starting with one season then onto the next during the year. In regions with low precipitation in metropolitan and mechanical regions, there is expanding rivalry for water utilizes, which require changes in water assets the executives. Lakes, water supplies and streams, which are most significant wellsprings of drinking water for the total populace, are helpless against contamination and debasement of water quality, especially to eutrophication. The new water assemblages of the world are by and large going through high paces of debasement prompting eutrophication. Connection coefficient estimates the closeness of the connection between two factors all at once. The upsides of connection coefficient closer to +1 or – 1, shows the likelihood of straight connection between the factors x and y. This examination endeavors to build up the idea of the direct connection between the factors and along these lines gives an instrument to forecast (Mulla et al., 2007; Kumar et al., 2005; P. Lilly Florence et al., 2012). Broad exploration has been done on factual examination to evaluate the surface water quality (Joshi et al., 2009) have surveyed the water quality attributes of Ganga in Haridwar, India utilizing Person's Correlation. Measurable examination of physico-chemical parameters of water has been accounted for from the various pieces of India by numerous specialists (Bhandari et al., 2008, Sharma et al., 2009; Indu et al.,2015). The prior has uncovered that the freshwater assets are under pressure and should be tended to shows for their reclamation for group of people yet to come. It is with this foundation, the current investigation on Ayyanakerelake has been embraced.

METHODOLOGY

Study area

Ayyanakere lake is an old water body and constructed by Rukumanda Raya who was the chief of Sakrayapatna and later renovated in 1156 AD during the Hoysala times (Figure 1). This large lake situated at the eastern base of Baba Budan range, 18 Km north east of Chikamagalur town, provides irrigation facilities to more than 1,500 hectares land. On a hillock adjacent to the lake, there is the Prassana Balleshwara shrine, Hoysal sculptures of Ganapathi, Surya, Krishna and Ambica. Ayyanakere lake area possess evergreen to deciduous forest types. The climate of the region is cool and dominated by many hillocks. The water body is completely surrounded by the small to larger hillocks with

perennial streams. This lake is situated at 13°14' 42"North latitude and 75° 04'46"Eastern longitude. This lake with small tributaries forms Vedavathiriver, which ultimately joins Bay of Bengal. Many hillocks surrounded the lake which forms the natural reservoir. It is a shallow lake and has an area of 15 sq km facilitating to about 1,500 hectare land for irrigation. The embankment forms the natural earth and stones with 1,700 feet long and 300 feet high at the rear slopes. The highest depth of the lake is 30 mtwith an average depth is 20 mt.

Statistical analysis

The obtained information during the examination time frame has been exposed to factual investigation. Karl Pearson's connection co-proficient has been determined to comprehend the connection between different physico-chemical boundaries. Karl Pearson's Coefficient of Correlation is broadly utilized numerical technique wherein the mathematical method is utilized to ascertain the degree and course of the connection between direct related factors. Pearson's strategy, prominently known as a Pearsonian Coefficient of Correlation, is the most widely utilized quantitative strategies by "r". In the event that the correlation between two factors X and Y is to be learned, then, at that point the accompanying equation is utilized:

$$r = \frac{\Sigma(X-\overline{X})(Y-\overline{Y})}{\sqrt{\Sigma(X-\overline{X})^2}\sqrt{(Y-\overline{Y})^2}}$$

Where, $\overline{X}$ - mean of X variable

$\overline{Y}$ - mean of Y variable

The coefficient of correlation always lies between ±1. The coefficient of correlation is represented by the following equation;

$$r = \sqrt{b_{xy} + b_{yx}}$$

RESULTS

Table 1 addresses the correlation acquired in station-I. The significant relations were addressed in bold letters. Air temperature of all the station has uncovered the critical positive relationship with the water temperature, water pH and nitrate substance. Along these lines an unmistakable relationship was seen among air and water temperatures. Air temperature has likewise uncovered the critical negative relationship with the electrical conductivity, total dissolved solids, dissolved oxygen, total acidity and sulfate. The air temperature, one of the autonomous parameter, shown the huge relationship with a large number of the parameters. The water temperature of the investigation stations has uncovered the huge positive relationship with the water pH as seen among the air temperature with water pH. Thus, a noticeable relationship can be seen among these boundaries in this investigation station. Like that of the air temperature, the water temperature has shown a huge negative relationship with the total dissolved solids and total alkalinity. The expand in the amount of the water temperature has drastically reduces the attention of the total dissolved solids and the total alkalinity. The water pH has exhibited the massive fine relationship with the nitrate and silica concentrations. Hence, the extend in the concentration of the water pH, i.e. the alkalinity of the water has significantly expand the concentrations of the nitrate and silica. The water pH additionally exhibited a sizeable but bad relationship with the electrical conductivity, total dissolved solids, complete hardness and total acidity. The concentration of the electrical conductivity has published the huge fine relationship with the total dissolved solids, calcium and whole hardness concentrations, while it showed the vast terrible relationship with the carbon dioxide concentration. Thus the make bigger in the awareness of the electrical conductivity can drastically enlarge the concentration of the complete dissolved solids, dissolved oxygen, calcium and complete hardness attention and limit the carbon dioxide concentration. The attention of the complete dissolved solids has showed a big fantastic relationship with the dissolved oxygen, total hardness and complete acidity. Hence,

the concentration of the total dissolved solids has big impact on the awareness of the dissolved oxygen, total hardness and complete alkalinity. The awareness of the turbidity recorded in the station-I has printed the wide spread advantageous relationship with the COD and potassium. The awareness of the dissolved oxygen has confirmed the significant fine relationship with the awareness of the calcium and potassium, while significant poor relationship with the concentration of the carbon dioxide. Hence the attention of the dissolved oxygen has tremendous effect on the carbon dioxide, calcium and potassium concentrations. The concentrations of the BOD and chloride have now not any relationship with any of the parameters recorded in the station-I. The awareness of the COD recorded in the station-I has revealed the large tremendous relationship with the potassium and silica concentrations. The concentration of the carbon dioxide has confirmed the significant terrible relationship with the total hardness concentration. The attention of the calcium has exhibited a great relationship with the magnesium, whole hardness and potassium concentrations.

The make bigger in the concentration of the calcium can substantially enlarge the concentration of the magnesium, total hardness and potassium concentrations. Similarly, the awareness of the magnesium has exhibited the high positive relationship with the total hardness, total alkalinity and potassium concentrations. The total acidity of the learn about station has a vast advantageous relationship with the nitrite and tremendous bad relationship with the nitrate concentrations. The concentrations of the phosphate and nitrate have revealed the full-size bad relationship with the nitrite concentration in the learn about station in the course of the period of study, while the concentration of the nitrite has the large nice relationship with the iron attention recorded in the learn about station. Many of the parameters also showed both wonderful or poor relationship among every other, but the relationship was once not statistically significant (Table1).Table 2 represents the Karl Pearson's correlation coefficient calculated among the physico-chemical parameters of the station-II. In this station, the quantity of the air temperature recorded has the significant high-quality relationship with the water temperature and water pH. Hence, the expand in the amount of the air temperature can notably extend the water temperature and water pH. The air temperature also confirmed the big bad relationship with the dissolved oxygen, sulphate and sodium. Therefore, the make bigger in the air temperature can substantially limit the concentration of the dissolved oxygen, sulphate and sodium concentrations.

Water temperature recorded in the station-II has published the considerable wonderful relationship with the water pH as observed in station-I and notably poor relationship with the electrical conductivity, dissolved oxygen and sodium. Further, the enlarge in the water temperature can reduce the concentration of the electrical conductivity, dissolved oxygen and sodium. The water pH has confirmed the widespread advantageous relationship with the chloride and silica concentrations, whilst huge poor relationship with the electrical conductivity. The electrical conductivity has the considerable advantageous relationship with the whole dissolved solids, turbidity and dissolved oxygen.

The increase in the awareness of the electrical conductivity has considerably amplify the attention of these parameters. The complete dissolved solids have showed the positive relationship with the complete alkalinity. The turbidity of the study station has the widespread fantastic relationship with the dissolved oxygen, COD, sulphate and potassium. The attention of the dissolved oxygen has printed the sizable fine relationship with the nitrite, sulphate and sodium. The amplify in the attention of the dissolved oxygen can significantly enlarge the concentrations of the nitrite, sulphate and sodium. The concentration of the BOD has showed the good fantastic relationship with the carbon dioxide and iron concentrations, whilst huge negative relation with the phosphate. The attention of the COD has confirmed the massive advantageous relationship with the magnesium, nitrite, potassium and silica concentrations. Hence, the attention of the COD has sizeable impact on the concentrations of magnesium, nitrite, potassium and silica. The attention of the carbon dioxide has published the substantial fantastic relationship with the sodium and iron concentrations and substantially poor relationship with the phosphate and potassium. The awareness of the calcium has printed the sizable advantageous relationship with the magnesium and complete hardness, in flip the magnesium awareness has showed the good sized effective relationship with the nitrite concentration.

The concentration of the phosphate has printed the huge terrible relationship with the sodium in turn has revealed huge fine relationship with the attention of the iron concentration. The correlation coefficient calculated amongst the physico-chemical parameters of the station-III has represented in Table 3 Similar to the station-I and II, in station-III also, the air temperature has the enormous high quality relationship with the water temperature and water pH. It has additionally showed the similar relationship with the concentration of COD and potassium. The air temperature has showed the poor relationship with the dissolved oxygen, total acidity and sulphate. Hence, the make bigger or decrease of the air temperature has big impact on the attention of the different parameters. The amount of the water temperature recorded in the study station has printed a good sized high quality relationship with the water pH and total alkalinity.

The water temperature additionally revealed the enormous bad relationship with the complete acidity. Therefore, all the parameters that have the relationship with air temperature additionally have significance on the parameters which have relationship with the water temperature. The water pH has the giant tremendous relationship with the carbon dioxide, total alkalinity and silica concentration. It also confirmed the big bad relationship with the electrical conductivity and total acidity. The electrical conductivity has showed the good sized superb relationship with the complete dissolved solids and large terrible relationship with the concentration of the carbon dioxide. The awareness of the turbidity has published the full-size nice relationship with the awareness of the nitrate and sulphate in the station-III. The amplify in the concentration of the turbidity has showed the great amplify in the concentration of the nitrate and sulphate in the find out about station.

The attention of the dissolved oxygen in the find out about station has printed the extensive high-quality relationship with the sulphate concentration and widespread negative relationship with the BOD, COD and carbon dioxide concentrations. The attention of the BOD has large nice relationship with the COD and potassium concentrations and in turn confirmed the extensive wonderful relationship with the carbon dioxide concentration. Hence, the concentrations of the dissolved oxygen, BOD and COD have the substantial effect on the enlarge or reduce of the different proximal related parameters. The attention of the chloride in the learn about station has published the giant tremendous relationship with the calcium, magnesium and the sulphate concentrations. Hence the attention of the chloride has the sizable effect on the amplify or decrease of the calcium, magnesium and sulphate concentrations. The calcium awareness in the study station has published the sizeable advantageous relationship with the magnesium concentration.

The total alkalinityhas confirmed the massive negative relationship with the total acidity. Similarly, the concentration of the whole acidity has exhibited the tremendous negative relationship with the potassium and silica concentrations. Hence, the quantity of the water pH has the increased importance on the best of the water. The concentration of the phosphate has confirmed the widespread nice relationship with the potassium, while the nitrate has confirmed the full-size fantastic relationship with the silica. Thus the physico-chemical parameters recorded in the find out about station-III have confirmed both fantastic or bad relationship among each other. The notably associated parameters have been discussed and referred to in the daring letters and the final parameters does not have the importance for the Karl Pearson's correlation calculation. Karl Pearson's correlation coefficient used to be calculated among the physico-chemical parameters of all the study stations. Table four represents the correlation received in station-IV. The considerable relationships have been represented in bold letters. The air temperature of the find out about station has published the large high quality relationship with the water temperature, water pH and whole alkalinity concentrations. The air temperature also confirmed the widespread terrible relationship with the awareness of the electrical conductivity. Thus the air temperature has the large effect on the attention of the other parameters.

The amount of the water temperature has printed the substantial tremendous relationship with the water pH, total alkalinity and silica concentrations. Thus the quantity of the water temperature has direct have an effect on on the attention of the whole alkalinity and silica and also on the attribute of water pH. Water pH recorded in the station-IV has printed the big superb relationship with the silica and poor relationship with the electrical

conductivity and complete dissolved solids. Hence, the water pH has the greater have an impact on on the attention of the silica, electrical conductivity and total dissolved solids. The awareness of the electrical conductivity has published the enormous effective relationship with the whole dissolved solids and turbidity. The amount of the whole dissolved solids has printed the enormous tremendous relationship with the awareness of the sulphate. The attention of the turbidity has the big nice relationship with the magnesium in the study station-IV. The awareness of the dissolved oxygen has the huge terrible relationship with the calcium and phosphate concentrations. Hence, the concentration of the dissolve oxygen has the inverse effect on the amplify or limit of the calcium and phosphate concentrations.

The awareness of the calcium awareness has exhibited a full-size nice relationship with the magnesium, phosphate and iron concentrations. The attention of the calcium has good sized and direct effect on the make bigger or decrease of the concentrations of magnesium, phosphate and iron. The attention of the sodium in the learn about station has published the giant superb relationship with the attention of the potassium. The expand in the attention of the sodium has a substantial effect in increasing the awareness of the potassium. Thus the physico-chemical parameters recorded in the find out about at station-IV have confirmed either effective or bad relationship among each other.

CONCLUSION

In general, the lake is oligotrophic, but seasonably tends to show the characters of eutrophication. Hence, it is suggested to maintain the status of the lake by enforcing the necessary mitigative environmental protection acts. Human habitation in the vicinity of the lake has to be checked and planned according to the needs only.An awareness among the public and farmers regarding the impact of pollution and conserving the quality of water should be created through mass media programmes and personal visits.

The farmers of the adjoining area should be educated for judicious utilization of chemical fertilizers to prevent the enrichment of the water through nitrogenous and phosphate derivatives. The use of pesticides should be avoided to the maximum extent possible. However, if necessary, the required dose of pesticides should be used against the target organism. This helps to reduce the pesticide load in the lake. The people residing in the vicinity should be advised strictly against washing the clothes and animals in the lake and also advised to take the alternative measures.The knowledge on the environmental degradation and conservation of the lake should be advised to the adjoining village people.The practice of encroaching the area by farmers of nearby villages for the irrigation during summer should be avoided.The surrounding hillocks offer an excellent panoramic view of the lake. Proper laws should be made to conserve the lake legally.

DECLARATION OF COMPETING INTEREST

The author declare that there is no competing interest.

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Table 1. Karl Pearson correlation coefficient calculated among the physico-chemical parameters of the station-I

	WT	pH	EC	TDS	Tur	DO	BOD	COD	CO_2	Cl	Ca	Mg	TH	T.Alk	T.Aci	PO_4	NO_2	NO_3	SO_4	Na	K	Fe	Si
AT	**0.831**	**0.769**	**-0.530**	**-0.625**	-0.447	**-0.563**	0.471	-0.122	0.257	-0.274	-0.209	0.009	-0.317	0.297	**-0.837**	0.346	**0.546**	-0.413	**-0.521**	0.159	-0.337	-0.294	0.293
WT		**0.716**	-0.488	**-0.624**	-0.274	-0.464	0.475	-0.021	0.222	-0.222	-0.004	0.188	-0.219	0.232	**-0.600**	0.085	0.326	-0.225	-0.470	-0.172	-0.202	-0.106	0.434
pH			**-0.710**	**-0.678**	-0.254	-0.403	0.333	0.174	0.455	-0.258	-0.015	0.118	**-0.521**	0.271	**-0.753**	0.103	**0.572**	-0.336	-0.352	-0.134	-0.039	-0.254	**0.538**
EC				**0.918**	0.455	**0.617**	-0.343	0.026	**-0.584**	0.250	**0.524**	0.434	**0.760**	0.090	0.460	0.251	-0.359	0.049	0.239	0.194	0.256	0.082	-0.317
TDS					0.377	**0.609**	-0.427	0.052	-0.472	0.261	0.474	0.403	**0.706**	0.093	**0.511**	0.147	-0.296	0.086	0.337	0.202	0.253	0.114	-0.328
TUR						0.460	-0.106	**0.582**	-0.361	0.105	0.477	0.465	0.464	-0.006	0.141	0.090	-0.193	-0.048	0.299	-0.343	**0.823**	0.020	0.124
DO							-0.479	0.205	**-0.688**	0.096	**0.610**	0.487	0.371	0.065	0.386	0.119	-0.318	0.158	0.020	-0.089	**0.511**	0.315	0.057
BOD								-0.263	0.352	-0.142	-0.145	-0.202	-0.120	-0.120	-0.274	-0.088	0.002	0.033	-0.305	-0.065	-0.192	0.017	-0.197
COD									-0.002	0.012	0.481	0.462	0.111	0.298	-0.258	0.002	0.236	-0.116	0.244	-0.368	**0.746**	-0.014	**0.548**
CO_2										-0.029	-0.387	-0.467	**-0.614**	-0.147	-0.176	-0.476	0.203	0.065	0.165	-0.038	-0.302	-0.119	0.113
Cl											-0.016	0.259	0.169	-0.043	0.344	0.054	-0.143	-0.086	0.210	-0.026	0.159	-0.364	-0.110
Ca												**0.784**	**0.560**	0.384	0.027	0.193	0.067	-0.177	-0.058	-0.262	**0.615**	0.114	0.309
Mg													**0.594**	**0.537**	-0.178	0.452	0.189	-0.356	-0.200	-0.142	**0.639**	-0.207	0.434
TH														0.279	0.211	0.394	-0.174	-0.120	-0.014	0.055	0.399	-0.073	-0.195
T.Alk															-0.408	0.331	0.411	-0.332	-0.440	-0.153	0.286	-0.279	0.490
T.Aci																-0.455	**-0.729**	**0.570**	0.364	-0.175	-0.062	0.422	-0.474
PO_4																	0.274	**-0.599**	-0.339	0.407	0.155	-0.492	-0.054
NO_2																		**-0.767**	-0.191	0.060	0.073	-0.440	0.475
NO_3																			0.139	-0.189	-0.197	**0.509**	-0.269
SO_4																				-0.049	0.056	0.143	-0.312
Na																					-0.427	-0.146	-0.398
K																						-0.131	0.485
Fe																							-0.235

All the parameters are in mg/L except water temperature (°C), pH, electrical conductivity (µmhos/cm) and turbidity (NTU). Significant at 0.5%

Table 2 Karl Pearson correlation coefficient calculated among the physico-chemical parameters of the station-II

	WT	pH	EC	TDS	Tur	DO	BOD	COD	CO_2	Cl	Ca	Mg	TH	T.Alk	T.Aci	PO_4	NO_2	NO_3	SO_4	Na	K	Fe	Si
AT	**0.808**	**0.726**	-0.499	-0.146	-0.411	**-0.719**	-0.161	0.105	-0.378	0.452	0.026	0.030	-0.186	0.111	-0.341	0.412	0.021	-0.424	**-0.557**	**-0.584**	0.073	-0.414	0.281
WT		**0.670**	**-0.536**	-0.229	-0.336	**-0.591**	-0.306	0.099	-0.377	0.445	-0.035	0.055	-0.198	0.194	-0.238	0.331	-0.150	-0.276	-0.450	**-0.518**	-0.082	-0.311	0.330
pH			**-0.540**	-0.486	-0.106	-0.491	-0.147	0.480	-0.389	**0.519**	0.146	0.175	-0.255	0.036	-0.492	0.224	0.144	-0.244	-0.324	-0.400	0.200	-0.282	**0.549**
EC				**0.697**	**0.539**	**0.592**	-0.332	-0.030	0.012	-0.245	0.430	0.365	0.368	-0.100	0.326	0.248	-0.092	0.339	0.406	0.276	0.355	0.041	-0.171
TDS					0.216	0.305	-0.462	-0.283	-0.140	-0.272	0.163	0.095	0.343	0.172	**0.544**	0.266	-0.068	0.087	0.085	0.143	0.114	-0.016	-0.303
TUR						**0.544**	-0.237	**0.574**	-0.311	-0.051	0.404	0.451	0.269	-0.026	0.214	0.062	-0.029	0.432	**0.615**	0.061	**0.527**	-0.020	0.157
DO							-0.167	0.124	0.085	-0.201	0.257	0.264	0.357	-0.181	0.475	-0.273	-0.284	**0.595**	**0.520**	**0.581**	0.152	0.137	-0.025
BOD								-0.037	**0.565**	-0.106	-0.397	-0.444	-0.369	0.142	-0.351	**-0.531**	-0.137	-0.104	0.043	0.185	-0.193	**0.523**	-0.245
COD									-0.420	0.432	0.485	**0.555**	0.059	-0.133	-0.059	0.075	-0.033	**0.504**	0.164	-0.257	**0.564**	-0.025	**0.714**
CO_2										-0.194	-0.321	-0.395	-0.188	0.238	-0.171	**-0.666**	-0.097	-0.132	0.203	**0.588**	**-0.516**	**0.524**	-0.364
Cl											0.235	0.253	-0.078	0.173	-0.193	0.014	-0.247	0.075	0.035	-0.070	0.111	0.006	0.409
Ca												**0.941**	**0.549**	-0.298	0.005	0.362	0.000	0.402	0.103	-0.063	0.376	-0.123	0.348
Mg													0.407	-0.287	0.104	0.336	-0.060	**0.521**	0.216	-0.181	0.335	-0.262	0.484
TH														-0.297	0.253	0.177	0.125	0.261	0.056	0.013	0.207	-0.081	0.063
T.Alk															-0.070	-0.292	-0.208	-0.221	0.026	0.113	-0.182	0.186	-0.282
T.Aci																-0.047	0.010	0.245	0.118	0.114	-0.114	-0.044	0.098
PO_4																	0.182	-0.078	-0.385	**-0.550**	0.473	-0.442	0.032
NO_2																		-0.251	-0.158	-0.388	0.134	-0.252	0.080
NO_3																			0.446	0.082	0.358	0.080	0.361
SO_4																				0.405	0.147	0.151	-0.036
Na																					-0.363	**0.623**	-0.388
K																						-0.239	0.292
Fe																							-0.266

All the parameters are in mg/L except water temperature (°C), pH, electrical conductivity (µmhos/cm) and turbidity (NTU). Significant at 0.5%

Table 3. Karl Pearson correlation coefficient calculated among the physico-chemical parameters of the station-III

	WT	pH	EC	TDS	Tur	DO	BOD	COD	CO_2	Cl	Ca	Mg	TH	T.Alk	T.Aci	PO_4	NO_2	NO_3	SO_4	Na	K	Fe	Si
AT	**0.773**	**0.598**	-0.306	-0.100	-0.415	**-0.684**	0.372	**0.555**	0.392	-0.054	0.020	0.039	-0.152	0.417	**-0.506**	0.463	-0.018	0.168	**-0.530**	0.214	**0.606**	0.232	0.203
WT		**0.749**	-0.297	-0.045	-0.224	-0.465	0.130	0.468	0.435	-0.075	0.207	0.298	-0.111	**0.626**	**-0.671**	0.144	0.064	-0.086	-0.284	0.042	0.435	0.256	0.484
pH			**-0.629**	-0.384	-0.160	-0.385	0.196	0.368	**0.654**	-0.075	0.231	0.280	-0.183	**0.664**	**-0.761**	0.146	0.350	-0.146	-0.324	0.046	0.449	0.393	**0.606**
EC				**0.536**	0.499	0.429	-0.138	-0.318	**-0.508**	0.441	0.277	0.274	0.232	-0.245	0.264	0.123	0.002	0.339	0.471	-0.362	0.052	-0.321	-0.202
TDS					0.263	0.249	-0.145	-0.194	-0.436	0.221	0.069	0.080	0.205	0.080	0.147	0.180	-0.209	0.094	0.195	-0.085	0.054	-0.351	-0.267
TUR						0.499	-0.086	-0.474	-0.166	0.382	0.343	0.425	0.248	0.311	-0.219	0.074	**0.506**	0.003	**0.694**	-0.240	0.097	0.112	0.182
DO							**-0.544**	**-0.803**	**-0.504**	0.304	0.311	0.308	0.359	-0.031	0.312	-0.126	0.050	-0.043	**0.640**	-0.299	-0.348	-0.105	0.020
BOD								**0.531**	0.322	0.119	-0.008	0.001	-0.061	0.034	-0.103	0.313	0.293	0.284	-0.356	0.114	**0.565**	0.132	0.181
COD									**0.571**	-0.215	-0.017	-0.005	-0.175	0.070	-0.165	0.115	-0.041	0.005	-0.483	0.076	0.332	0.102	0.160
CO_2										-0.293	-0.190	-0.167	-0.471	0.295	-0.441	-0.332	0.396	-0.030	-0.132	0.099	0.204	0.454	0.351
Cl											**0.574**	**0.506**	0.476	0.230	-0.047	0.394	0.145	0.343	**0.500**	-0.186	0.446	0.155	-0.102
Ca												**0.956**	0.484	0.356	-0.173	0.371	0.261	0.022	0.215	-0.383	0.381	0.117	0.379
Mg													0.452	0.456	-0.317	0.385	0.292	-0.075	0.221	-0.402	0.396	0.108	0.495
TH														0.193	0.081	0.203	0.095	0.069	0.359	0.167	0.023	-0.165	0.024
T.Alk															**-0.738**	0.233	0.388	-0.132	0.178	0.070	0.481	0.423	0.470
T.Aci																-0.178	-0.472	0.132	0.104	-0.008	**-0.517**	-0.347	**-0.555**
PO_4																	-0.170	0.142	-0.273	-0.124	**0.555**	0.003	0.048
NO_2																		0.308	0.230	-0.070	0.418	0.308	**0.632**
NO_3																			0.004	0.051	0.274	-0.042	-0.219
SO_4																				-0.203	-0.191	0.067	-0.089
Na																					-0.144	0.149	-0.186
K																						0.261	0.424
Fe																							0.223

All the parameters are in mg/L except water temperature (°C), pH, electrical conductivity (µmhos/cm) and turbidity (NTU). Significant at 0.5%

Table 4. Karl Pearson correlation coefficient calculated among the physico-chemical parameters of the station-IV

	WT	pH	EC	TDS	Tur	DO	BOD	COD	CO_2	Cl	Ca	Mg	TH	T.Alk	T.Aci	PO_4	NO_2	NO_3	SO_4	Na	K	Fe	Si
AT	**0.842**	**0.554**	**-0.594**	-0.302	-0.357	-0.318	0.100	0.359	-0.364	-0.045	0.246	0.114	-0.204	**0.619**	-0.235	0.445	-0.217	-0.061	-0.251	-0.231	-0.043	0.168	0.313
WT		**0.711**	-0.489	-0.313	-0.123	-0.096	0.101	0.419	-0.471	-0.075	0.085	0.303	-0.213	**0.606**	-0.466	0.160	0.066	0.019	-0.338	-0.235	-0.009	0.059	**0.539**
pH			**-0.611**	**-0.601**	-0.104	-0.250	0.016	0.483	-0.278	-0.202	-0.025	0.285	-0.423	0.409	-0.470	-0.016	0.013	-0.050	-0.368	0.014	0.054	-0.001	**0.602**
EC				**0.800**	**0.534**	0.123	0.325	-0.372	0.268	-0.104	0.248	0.343	0.405	-0.151	0.040	0.185	0.354	0.329	0.388	-0.050	-0.002	0.028	-0.193
TDS					0.364	0.137	0.061	-0.335	0.214	-0.073	0.328	0.420	0.498	-0.199	-0.022	0.385	0.169	0.431	**0.517**	-0.241	-0.137	-0.098	-0.243
TUR						-0.112	0.101	-0.032	-0.028	0.177	0.237	**0.503**	0.113	0.053	-0.254	0.115	0.406	0.309	0.144	-0.148	-0.115	0.064	0.387
DO							-0.379	-0.392	0.263	-0.047	**-0.591**	-0.258	0.153	-0.338	0.081	**-0.637**	0.134	0.025	0.066	-0.078	0.128	-0.222	-0.374
BOD								0.110	-0.296	-0.125	0.313	0.099	0.091	0.467	-0.059	0.369	0.261	-0.078	-0.141	0.130	0.039	0.176	0.210
COD									-0.392	0.041	-0.111	0.148	-0.351	-0.075	-0.298	0.029	0.152	-0.319	-0.148	-0.213	-0.091	-0.033	0.404
CO_2										0.008	-0.050	0.071	0.017	-0.324	0.403	-0.145	-0.130	-0.202	0.165	0.125	0.217	-0.187	-0.478
Cl											-0.187	-0.330	-0.188	0.030	0.138	-0.086	0.059	-0.479	0.027	0.145	0.148	-0.179	-0.054
Ca												**0.516**	0.247	0.486	-0.222	**0.793**	-0.162	0.334	0.120	-0.103	-0.258	**0.511**	0.126
Mg													0.084	0.195	-0.463	0.402	0.323	0.484	0.119	-0.225	-0.111	-0.051	0.494
TH														-0.140	0.007	0.159	-0.295	0.191	0.099	-0.092	-0.210	0.288	-0.181
T.Alk															-0.390	0.476	-0.023	0.078	-0.164	-0.170	-0.136	0.291	0.373
T.Aci																-0.280	-0.218	-0.287	-0.325	0.488	0.496	-0.053	-0.479
PO_4																	-0.171	0.248	0.283	-0.244	-0.328	0.323	0.093
NO_2																		0.217	0.112	-0.027	0.115	-0.168	0.390
NO_3																			0.226	-0.123	-0.118	0.086	0.242
SO_4																				-0.252	-0.150	-0.166	-0.103
Na																					**0.797**	-0.115	-0.074
K																						-0.255	0.030
Fe																							-0.080

All the parameters are in mg/L except water temperature (°C), pH, electrical conductivity (µmhos/cm) and turbidity (NTU). Significant at 0.5%

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RESEARCH ARTICLE

Valproic acid Mediated Drug Resistant Epilepsy Can Be Overcome by Combining the Effect of Glycyrrhizin and Valproic acid via on *In vitro* Co-Culture of Astrocytes and Microglia

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ABSTRACT

The contribution of glial cells, mainly astrocytes and microglia, to the patho physiology of epilepsy is increasingly appreciated. Glia plays a pivotal role in the initiation and maintenance of the central nervous system (CNS) immune response and neuronal metabolic and trophic supply. Recent clinical and experimental evidence suggests a direct relationship between epileptic activity and CNS inflammation, which is characterized by accumulation, activation, and proliferation of microglia and astrocytes. Concomitant glia-mediated mechanisms of action of several antiepileptic drugs (AEDs) have been proposed. Hannes Dambach *et al*., 2014, he was hypothesised that the CNS inflammation is defined by a disruption of glial cell activities. In M5 and M30 co-cultures, Valproic acid, Pheytoin, and Gabapentin were found to produce substantial microglial activation, a characteristic feature of inflammation. Concerning the direct link between CNS inflammation, which was caused by valproic acid, and seizures. But just a few research have examined how they affect glial cells specifically. Using a co-culture paradigm of astrocytes and microglia, Hence, Present work were wanted to see first time, how is our used AEDs of Valproic acid affected glial survival, the gap junctional network, microglial activation, and cytokine expression and these findings were focused to Co-administration of glycyrrhizin plus Valproic

acid to overcome .this limitation via In-vitro evaluating astrocytes and microglia activation in M5 and M30 astrocytes and microglia co-cultures for an modulation of immunity and inflammatory response in drug-resistant epilepsy. Our result indicates that AEDs of Valproic acid with anti-inflammatory glial characteristics are helpful for seizures brought on by prolonged brain inflammation, which were prevented by the simultaneous administration of Valproic acid and Glycyrrhizin.

Keywords: Valproic acid**,** Glycyrrhizin, Astrocytes, Microglia, Cellular viability and Epilepsy

INTRODUCTION

Epilepsy is a chronic neurologic illness that affects more than 70 million people all over the world. Despite the availability of over 20 Anti-Seizure Drugs (ASDs) for the symptomatic treatment of epileptic seizures, approximately one-third of epilepsy patients experience seizures that are resistant to pharmacotherapy due to CNS inflammation. Patients with Drug-Resistant Epilepsy (DRE) have a higher risk of death, injury, psychosocial dysfunction, and a lower quality of life, making the discovery of more effective medicines a critical clinical need [1-4]. The issue is complicated by the many types of epilepsy and seizures, as well as the intricate temporal patterns of refractoriness. Furthermore, the basic mechanisms of DRE remain a mystery. Immunity and inflammation are now considered major aspects of the pathobiology of epilepsy, because to extraordinary advances in immunology. Both experimental seizure animal models and epileptic patients have shown activation of inflammatory processes in brain tissue. Both in clinical and experimental settings, anti-inflammatory and immunotherapies exhibited strong anticonvulsant properties. According to the accumulating findings, regulation of immune and inflammatory processes could serve as novel specialised targets for achieving possible anticonvulsant benefits in epileptic patients, particularly those with DRE.

The formation of an early immune response is mediated by pro-inflammatory and anti-inflammatory cytokines, chemokines, and prostaglandins [4]. Some specific inflammatory mediators and their corresponding receptors are up-regulated in epileptic brain tissue, according to experimental and clinical discoveries over the last decade [4]. Interleukin-6 (IL-6), TNF-, and IL-1 are the most well-known cytokines [4]. In both experimental seizure animal models and human epileptic tissue from DRE patients, activation of the IL-1 type 1 receptor/Toll-like receptor (IL-1R/TLR) signal pathway has been shown to be a significant factor contributing to seizure activity [4]. These Endogenous substances such as pro-inflammatory cytokines or danger signals, as well as ligands associated with infections, can activate this signal pathway [4]. IL-1 and High Mobility Group Box 1 (HMGB1) were produced and released at the same time by astrocytes and microglia in the brains of mice with epilepsy. Notably, proconvulsant medicines appear to cause a rapid release of HMGB1 from neurons even before a seizure occurs, and this appears to be implicated in the precipitation of seizures [4]. Valproic acid is an anticonvulsant (or anti-epileptic) medicine. It's not fully understood how this medicine works for treating bipolar disorder. However valproic acid is thought to reduce or prevent manic episodes by increasing the amount of a chemical called Gamma-Amino Butyric Acid (GABA) in the brain [2]. It's been shown to cause increased adverse effects including CNS inflammation due to astrocyte and microglia activation caused by HMGB1 over expression.

The contribution of glial cells, mainly astrocytes and microglia, to the patho physiology of epilepsy is increasingly appreciated. Glia plays a pivotal role in the initiation and maintenance of the central nervous system (CNS) immune response and neuronal metabolic and trophic supply. Recent clinical and experimental evidence suggests a direct relationship between epileptic activity and CNS inflammation, which is characterized by accumulation, activation, and proliferation of microglia and astrocytes. Concomitant glia-mediated mechanisms of action of several antiepileptic drugs (AEDs) have been proposed. CNS inflammation is characterized by a disturbance of glial cell functions. Strong microglial activation, a typical hallmark of inflammation, was induced by VPA, PHE and GTA (Gabapentin) in M5 and continued in M30 co-cultures [5]. With regard to the direct relation between CNS

inflammation and seizures and valproic acid produced CNS inflammation. However, just a few studies have looked into their direct impact on glial cells. In an in vitro astroglia /microglia co-culture paradigm, Hence, Present work will be wanted to see first time, how will be our used AEDs of Valproic acid affected glial survival, the gap junctional network, microglial activation, and cytokine expression and will be focused to Co-administration of glycyrrhizin plus Valproic acid (Shown Fig-1) to overcome .this limitation via In-vitro evaluating astrocytes and microglia activation in M5 and M30 astrocytes and microglia co-cultures for an modulation of immunity and inflammatory response in drug-resistant epilepsy.

Glycyrrhizin (glycyrrhizic acid) Shown Fig-1, a bioactive triterpenoid saponin ingredient of Glycyrrhiza glabra with anti-inflammatory, antioxidant, antibacterial, and antiaging effects, is a traditional medication. It's a well-known pharmacological inhibitor of high mobility group box 1 (HMGB1), a ubiquitous protein that acts like a proinflammatory cytokine [6-11]. When produced extracellularly, HMGB1 has been linked to a variety of inflammatory disorders, mostly by activating intracellular signalling by binding to the receptor for advanced glycation end products (RAGE) and toll-like receptor 4 (TLR4) (TLR4).

MATERIALS AND METHODS

Molecular Docking

The target protein HMGB1 structure is retrieved from Pdb database based on the sequence similarity with protein structure database (2RTU). The target protein has two domains of which HMG (high mobility group) box of 71 residue domain in an HMG-box domain structure at 6-78 amino acid residues in 3D protein structure. The active site amino acids of Asp8, Arg13, Gly14, Met16, Ser17, Ala20, Phe21, Gln24, Glu28, Glu43, Lys46, Ser49, and Lys68 are used for ligand binding sites. The target protein is docked with Glycyrrhizin ligand structure using Autodock4.2 using default parameters.

In vitro astroglia/microglia co-culture paradigm

Cell culture

Primary glial cell cultures were prepared from brains of postnatal (P0–P2) Wistar rats according to Faustmann *et al* (12-15). Cultures were maintained in a 5% CO2, 95% air atmosphere at 37°C, and nearly 100% relative humidity. Adherent astroglial cells reached confluency after 4–5 days in Dulbecco's modified Eagle's medium (DMEM) culture medium. Afterwards microglial cells and oligodendroglia were separated from the astroglial surface by shaking the cell culture flasks. Based on the intensity of the shaking, the fraction of microglial cells remaining in the co-cultures varied between 5% (M5; representing physiologic microglial percentage in the brain tissue) and 30% (M30; representing microglial percentage under cerebral inflammatory conditions). To determine whether co-cultures contained a 5% or a 30% fraction of microglial cells, immunocytochemical staining followed by cell counting will be performed as described below. For the individual experiment, the confluently grown co-cultures were removed from the culture dishes by trypsin ethylenediaminetetraacetic acid (EDTA) (0.1%) after the shaking step mentioned and passaged onto poly-L-lysine glass cover slips (12 mm2) with a density of 60,000 cells per cover slip. Alternatively, co-cultures were passaged onto poly-L-lysine–covered 96-well plates reaching a density of 10,000 cells per well.

Drug application

We were used efficient concentrations of the testing antiepileptic substances. Such as Valproic acid (VPA), Glycyrrhizin (GLYZN) and Co-admistered VPA+GLYZN in the concentration of 10, 25, 50, and 100 µg/ml] were dissolved in sterilized distilled H2O were diluted in dimethyl sulfoxide (DMSO)/distilled H2O at a final concentration of DMSO <1%. Co-cultures were incubated with VPA, Glycyrrhizin, and Co-administration of VPA+GLYZN for 24 h in a 5% CO2, 95% air atmosphere at 37°C [12-15].

MTT-Viability assay

For investigation of the concentration-dependent influence of the testing antiepileptic substances on the viability and proliferation of the co-cultures, the Cell Proliferation Kit I (Roche Diagnostics, Mannheim, Germany) were used according to the manufacturer's protocol. The assay were based on the cleavage of the yellow-colored tetrazolium salt MTT (-3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide) to purple-colored formazan crystals, by enzymes of the endoplasmatic reticulum, in metabolically active cells only. Moreover, the amount of formazan dye were proportional to the amount of metabolically active cells in culture. The absorbance were finally measured with the microplate reader (Bio-Rad 550, Munich, Germany) set to 570 nm. The absorbance that was revealed directly correlated with the metabolically active number of glial cells [12-15].

Immunocytochemistry

To investigate the state of microglial activation, co-cultures were washed with phosphate-buffered saline (PBS) and fixed in 100% ethanol. To avoid unspecific antibody binding, blocking with PBS containing 10% bovine serum albumin (BSA) and 1% horse serum (HS) was applied. Microglial cells were labeled using a monoclonal antibody to the ED1 marker 1:250 in blocking solution (Serotec MCA 341R, Eching, Germany). After 12 h, cover slips were washed in PBS containing 10% BSA and incubated with the secondary antibody Alexa red 568 anti-mouse (Invitrogen, Karlsruhe, Germany) 1:1,000 in PBS containing 10% BSA. To count the total glial cell number, labeled cells were counterstained with DAPI (4, 6-diamidino-2-phenyl-indol) to visualize the nuclei. Cover slips were embedded in a Pro-Long antifade kit (Molecular Probes, Leiden, The Netherlands). The subsequent analysis were carried out under the Axiovert 35 microscope. The percentage of microglia to the total number of glial cells were formed by calculating the ratio of the number of ED1-stained microglia counted to the total number of DAPI-labeled glial cells. Furthermore, to ensure that the DAPI-labeled glial cells consisted mainly of astrocytes and microglia, a control staining with the astrocyte marker (glial fibrillary acidic protein), oligodendrocyte marker (20,30-cyclic-nucleotide phosphodiesterase) and neuronal marker (neuronal nuclei) were performed. According to Faustmann *et al*., microglial phenotypes were classified as resting ramified (RRT), intermediate (INT), or activated, rounded phagocytic (RPT) type. For each field of view (n), the fraction of microglia subpopulation (RRT, INT, and RPT) to the entire microglial cell population were defined. For the M5/M30 control as well as testing AED treatments, the mean percentage of all visual fields together for microglial RRT and RPT were calculated. Finally, the mean percentage of each microglia phenotype (RRT, RPT) in the control group were said on set to 1. Then the percentage of phenotypes in testing AED-treated cultures were normalized to 1. Controls were obtained from a pooled control group of the same set of staining [12-15].

RESULTS AND DISCUSSION

The ligand structure was strongly interacted with active site amino acids of our targeted protein HMGB1 by forming 4-5 hydrogen bonds and interaction energy of -10.19kcal/mol, inhibitory constant of 34.09nM with the binding amino acids of Arg13: HE:57:O, Trp52: HE1: 31:O, Ala20:HN: 33:O, Lys15:HZ3: 58:O Shown Table-1 and Figure-2-3. Hence, the proposed Glycyrrhizin was suitable for targeting inhibition of HMGB1 mediated microglia activation. The astrocytes, co-cultured with 5% microglia (M5 co-cultures), demonstrated a dose-dependent, substantial reduction in glial viability after incubation with GLYZN (Glycyrrhizin) and Co-adminstration of VPA (Valproic acid) and GLYZN (Glycyrrhizin) (Shown Fig-4-5A,B,C,D). Additionally, at every dose that was looked at, VPA (Valproic acid) caused a highly substantial activation of the microglia. Additionally, astrocytes co-cultured with 30% microglia (M30 co-cultures) showed a dose-dependent significant reduction in glial viability after incubation with co-adminstration of VPA+GLYZN, and significantly decreased the amount of activated microglial cells and increased the overall number of inactivated microglia. Finally, we looked at how co-administration of VPA+GLYZN affected survivability at all dosages.

Disrupted glial cell activities are a hallmark of CNS inflammation. The direct correlation between CNS inflammation and seizures is caused by strong microglial activation, a characteristic feature of inflammation, which was generated by VPA (Valproic acid) in M5 and persisted in M30 co-cultures, The co-adminstration of Valproic acid (VPA) and glycyrrhizin (GLYZN), which showed a considerable deactivation of microglia after the M30 co-cultures were incubated, had the opposite effect.

CONCLUSION

Our result indicates that AEDs of Valproic acid with anti-inflammatory glial characteristics are helpful for seizures brought on by prolonged brain inflammation, which were prevented by the simultaneous administration of Valproic acid and glizin (Glycyrrhizin). Future, the goal of the current study will be determined for the first time how the use of AEDs like Valproic acid will influence glial survival, the gap junctional network, microglial activation, and cytokine expression, particularly TNF and HMGB1. This restriction will be accomplished through the development of glycyrrhizin-decorated Valproic acid liposomes, which target HMGB1 inhibition and control immunity and inflammation in drug-resistant epilepsy.

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Table-1: Docking Scores of Glycyrrhizin and HMGB1

Binding Energy	Inhibitory constant	No of h Bonds	Amino Acids in Active Site
-10.19	34.09nM	5	Arg13: HE:57:O Trp52: HE1: 31:O Ala20:HN: 33:O Lys15:HZ3: 58:O

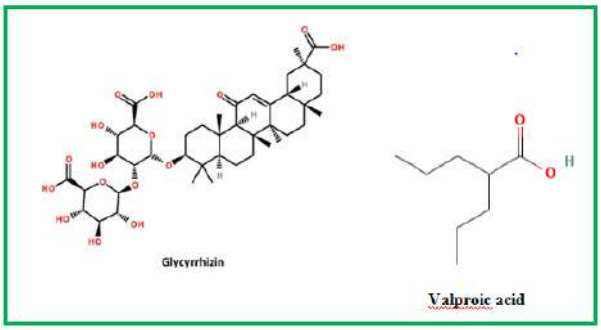

Figure 1: Chemical Structure of Glycyrrhizin and Valproic Acid.

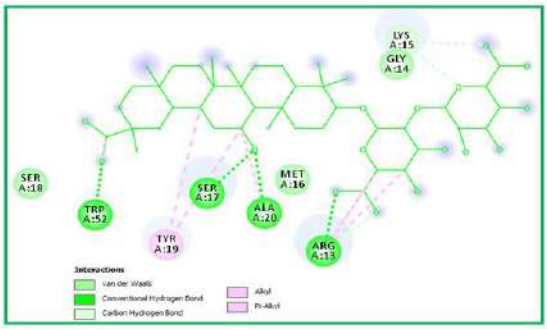

Figure 2: Shown The Ligand-Protein Interactions of Glycyrrhizin in the active site of HMGB1

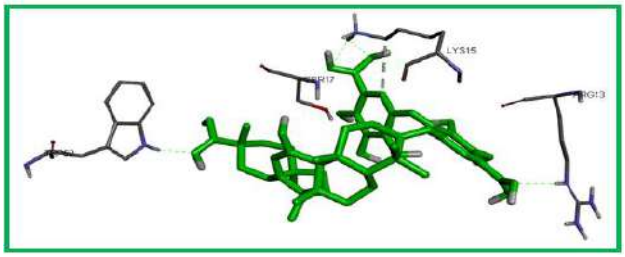

Figure 3 Shown the 3D-Vissualization of The Ligand-Protein Interactions of Glycyrrhizin in the active site of HMGB1

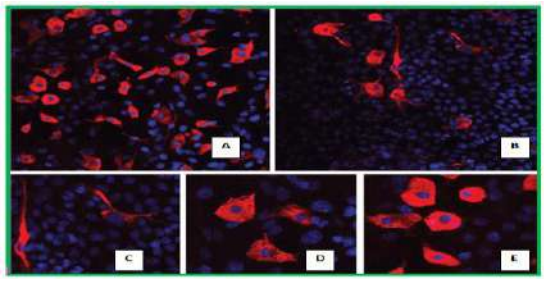

Figure 4. shows the immunocytochemical labelling of microglial cells (red) with the monoclonal antibody to ED-1 and counterstained with DAPI to see the nuclei (blue) of the total number of glial cells. (A) Astrocytes co-cultured with 30% microglial cells. (B) Astrocytes cocultured with 5% microglial cells. (C,D,E) classification of the three main microglial phenotypes (resting ramified type (C), intermediate (D), and active, round phagocytic type) (E).

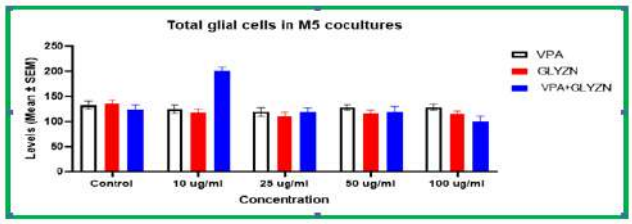

Figure 5A

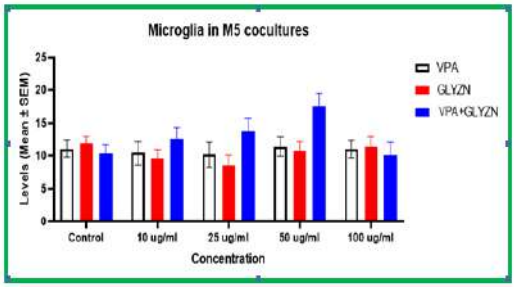

Figure 5B

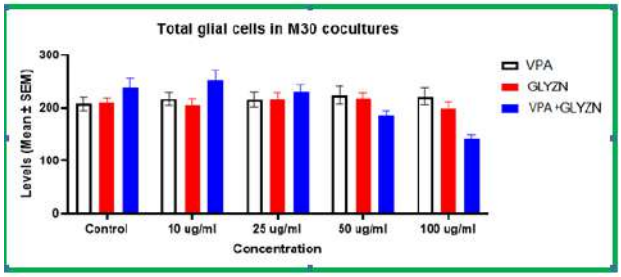

Figure 5C

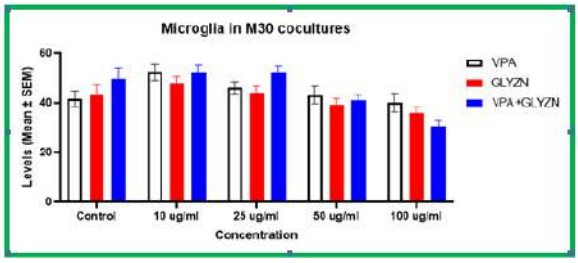

Figure 5D

Figure-54 A,B,C,D shown MTT Viability assay of M5 and M30 co-cultures after concentration-dependent incubation [10, 25, 50 and 100 ug/ml] with VPA (Valproic acid), GLYZN (Glycyrrhizin) and Co-adminstration of VPA+GLYZN for 24 h. Graphs show mean of n = 10 independent experiments for M5 and n = 12 independent experiments for M30 co-cultures. The viability of M5 as well as M30 co-cultures was dose dependently reduced after incubation with GLYZN and Co-adminstration of VPA+GLYZN after 24 h. The combination (C0-administration) of VPA+GLYZN had shown the inhibition effect of both M5 and M30 Co-cultures. In case of M5 the total glia cell shown the inhibition effect attained at the drug concentration of 10 μg/ml, where is an microglia shown its effect at 50 μg/ml and comparatively less at 10 & 25 μg/ml. In case of M30, the inhibition effect showed only at the concentration of 10 & 25 μg/ml but other concentration no effects.

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RESEARCH ARTICLE

Standardisation of Seed Priming Treatments with Herbal Extract to Enhance Seed Quality in Groundnut (*Arachis hypogaea*) Var. VRI 8

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ABSTRACT

Groundnut is one of the significant food and oil crops which is cultivated in tropical and sub-tropical region. It is the world's fourth largest source of edible oil and third largest source of vegetable protein. Uniformity in seedling emergence of direct seeded crops has a significant influence on overall seed quality. Slow emergence produces weaker seedlings that are more susceptible to disease. Priming permits the metabolic processes required for germination to take place without germination taking place. Seed priming is an effective strategy to enhance rapid and uniform emergence as well as high vigour, resulting in improved seedling establishment. The groundnut var. VRI 8 seeds were soaked at 2, 4, 6 and 8 hours of duration at 1, 3, 5 and 10 % concentrations in addition to tap water of holy basil leaf extract and unprimed seed as control. The study revealed that, the seeds primed with holy basil leaf extract 5 % for 6h were recorded the higher imbibition rate (79.42 per cent), speed of germination (7.9), germination percentage (94 per cent), longer root length (18.2 cm), shoot length (15.2 cm), higher dry matter production (3.82) and vigour index (3140). But the unprimed control seeds recorded lower imbibition rate (65.17),speed of germination (3.0), germination percentage (72 per cent), shorter root length (11.4 cm), shoot length (10.9 cm), lower dry matter production (1.95 g)and vigour index (1809).

Keywords: Groundnut, Seed priming and Holy basil leaf extract.

INTRODUCTION

Groundnut (*Arachis hypogea* L.) is one of the significant oilseed crops, which belongs to the family Fabaceae. It is a self-pollinated crop cultivated in tropical and subtropical parts of the world. Other names for groundnut include peanut, earthnut, potato bean, manila nut, goober, pinda, and monkey nut. Groundnut is commonly referred to as "the king of oilseeds." Groundnut is said to be a native of Brazil. It is an essential food source due to its high protein

(20-30 %) and fat content (45-55 %), with some varieties possessing more than 50 % edible oil [1]. It is also rich in calcium, magnesium, iron, vitamin E and riboflavin. Groundnut is identified as an excellent fodder for livestock and industrial raw material. Because of its numerous applications, groundnut is a good cash crop for both local and international markets in a number of developing and established countries. Seedling emergence and establishment are the two most important requirements for a successful seed production programme because they allow for not only uniformity in field stand but also full exploitation of a varieties yield potential. Good quality seeds with quick and uniform field emergence are required for enhanced seed yield and quality. Slow emergence produces low vigour seedlings that are more susceptible to disease. To shorten the period between sowing and seedling emergence, seed priming have been practiced. Seed priming is a pre-sowing seed treatment that permits the seeds to be hydrated and proceed through the initial stage of germination while preventing radical protrusion through the seed coat [2]. The advantages of priming include enhanced germination rate, consistency in seedling emergence in a wide range of conditions, and improved seedling vigour and development. Leaf extracts have been found to perform better in crop growth and yield [3]. Therefore, the study was carried out to determine the effect of different concentrations and soaking durations of pre-sowing seed treatment with holy basil leaf extract on seed quality in Groundnut (*Arachis hypogaea*) var. VRI 8.

MATERIALS AND METHODS

Groundnut (*Arachis hypogaea*) var. VRI 8seeds obtained fromThe Research Station, TNAU, Vridhachalam served as the base material for the study. The Laboratory experiment were conducted at Seed Science and Technology Laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India (located at 11°24′N latitude and 79°44′E longitude with an altitude of + 5.79 mts above mean sea level).The experiment was carried out with four replications in factorial completely randomized block design. Groundnut var. VRI 8 seeds were surface sterilised by soaking in 0.1 percent mercuric chloride solution for three minutes, then thoroughly washed with distilled water and then dried. These surface sterilised seeds were primed with different concentrations of holy basil leaf extract i.e. 1, 3, 5 and 10 % in addition to tap water and four different soaking durations i.e. 2, 4, 6 and 8 hours and unprimed seed as control to study on groundnut seed germination, growth and seedling vigour.

Preparation of leaf extract

Make the powder of shade dried leaves of *Ocimum sanctum* L. using electric grinder. One gram of leaf powder were weighed and dissolved in 100 ml of distilled water to make 1 per cent holy basil leaf extract and these solutions were maintained at room temperature for 48 hours. The leaf extracts were filtered through two layers of muslin cloth after 48 hours to eliminate unwanted material and leaf debris. Similarly for 3 %, 5 % and 10 % leaf extract solutions were prepared using above method. Seeds were soakedin different concentrations of holy basil leaf extracts at room temperature for different soaking hours. After priming, the seeds were removed from the solutions, washed in water, and shade dried at room temperature before being tested for the seed quality characteristics listed below.

Imbibition rate (%)

Twenty-five primed seeds were selected from each replication and placed in petri plates that were inner layered (two layers) with wet germination paper and maintained at room temperature for 24 hours. Every two hours, the seed weight increased was measured. The imbibed seeds were removed and cleaned with tissue paper to eliminate any remaining water droplets before being weighed on a top pan balance for their ultimate weight. The imbibition rate was estimated using the weight difference, and the mean was represented in percentage [4].

Speed of germination

$$\textbf{Imbibition rate}\ (\%) = \frac{\boldsymbol{Final\ weight - Orginal\ seed\ weight}}{\boldsymbol{Orginal\ seed\ weight}} \boldsymbol{X\ 100}$$

From the first to the tenth day of sowing, germinated seedlings from each replication of treatments were counted daily. The speed of germination was determined using the following method and represented in numbers based on the quantity of seeds germinated on each day [5].

$$\textbf{Speed of germination} = \frac{X_1}{Y_1} + \frac{X_2 - X_1}{Y_2} + \ldots\ldots\ldots\ldots\ldots + \frac{X_n - (X_n - 1)}{Y_n}$$

Where

X_1 - Number of seeds germinated at first count

X_2 - Number of seeds germinated at second count

X_n - Per cent germination on 'nth' day

Y_1 - Number of days from sowing to first count

Y_2 - Number of days from sowing to second count

Y_n - Number of days from sowing to 'nth' count

Germination percentage (%)

The germination test was carried out according to ISTA standards, with four replications each of 100 seeds in sterilised sand medium. The normal seedlings developed after 10 days were counted and expressed in percentage [6].

Root length (cm)

The root length was measured from the collar region to the primary root tip. The average root length was measured in centimeters (cm).

Shoot length (cm)

The shoot length was measured from the collar region to the tip of the primary leaf. The mean shoot length was calculated and represented in centimeters (cm).

Dry matter production (g)

The seedlings chosen for root and shoot length observation were shade dried for 24 hours and placed a seedlings in a butter paper bag and then dried in a hot air oven at 80°C for 24 hours. Then cooled in a desiccator for 1 hour, weighed, and expressed as gram per 10 seedlings.

Vigour Index

The following formula was used to calculate the vigour index values, and the mean values were represented as whole numbers[7]. Vigour Index = Germination (%) × seedling length (cm)

Statistical Analysis

The data were analysed statistically adopting the procedure described by [8].

RESULTS AND DISCUSSION

Seed quality is one of the most important factors influencing crop production potential, it should reach farmers in good condition. When compared to other inputs, quantity of seed used is low, yet it is the major tool for food security and it determines the agricultural output. Using high-quality seeds can increase agricultural production by 20-25 percent[9]. The unfavourable environmental conditions eventually result in crop failure, which raises agricultural production costs and puts impoverished farmers in debt. Among the several methods of solution, seed priming is a simple and effective method for successfully establishing crops. The controlled hydration of seed prevents germination while allowing for pre-germinative physiological and biochemical changes. Seed priming helps in better seedling development and ensures the uniformity in germination[10]. Hence a study was formulated

to evaluate the effect of seed priming with holy basil leaf extract on seed quality in Groundnut (*Arachis hypogaea*) var. VRI 8. The fresh seeds of Groundnut var. VRI 8were soaked in four concentrations (1, 3, 5 and 10 %) of holy basil leaf extract in addition to water for 2, 4, 6 and 8 hours and unprimed seed as a control. It revealed that the seeds soaked in holy basil leaf extract 5 % for 6 h was able to germinate earlier. The seeds primed with holy basil leaf extract 5 % for 6 h produced higher higher imbibition rate (79.42 per cent), speed of germination (7.9), germination percentage (94), root length (18.2), shoot length (15.2), dry matter production (3.82) and vigour index (3140) when compared to unprimed seed and other treatments (Table. 1, 2 and 3). Holy basil leaf extract contain saponin-like substances that serve as a precursor of gibberellic acid and minerals, particularly zinc, may have combined synergistically with amino acids and tryptophan to synthesize indole acetic acid (IAA) in seed and it stimulate the seed germination[11]. Further more, the leaf extract contains flavonoids, triterpenoids, and tannins and with antioxidant properties. The improvement in germination by botanical leaf extracts could be attributed to cell activation, which leads to an increase in mitochondrial activity, which contributes for the formation of higher energy compounds and essential biomolecules that are made available during the early stages of germination [12]. Many researchers suggests that plant extracts consists large number of microbes that helps in the plant growth because they secrete plant growth promoters (auxins, gibberellic acid, cytokinin and abscisic acid) and enhance seed germination. The increase in seedling root and shoot length observed by holy basil leaf extract seed priming treatment is most likely due to increased metabolic activity caused by cell elongation and multiplication (Kaiser *et al.,* 2005)[13].The basil leaf powder contains vitamin C and A as well as minerals like calcium, zinc, and iron, as well as many other phytonutrients, may account for its influencing impact [14].The macro and micro nutrients found in the leaf powder promote the invigorating impact of botanical treatments[15]. Several scientists have shown that eco-friendly and inexpensive botanicals enhance seed viability and vigour[16] [17].

CONCLUSION

From the present study, it is revealed that the seed priming with holy basil leaf extract 5 % for 6 h recorded higher values for germination percentage, root length, shoot length, dry matter production and vigour index when compared to other treatments. It is recommend to enhance seed and seed quality parameters of groundnut.

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Table 1. Effect of holy basil leaf extract seed priming on imbibition rate (%) and speed of germination of Groundnut var. VRI 8

Priming agent (A)	Imbibition rate (%)					Speed of germination				
	Soaking duration in h (D)					Soaking duration in h (D)				
	2	4	6	8	Mean	2	4	6	8	Mean
T_1 - Hydropriming	67.43	71.52	72.82	67.94	69.93	3.9	5.8	6.1	5.3	5.3
T_2 – Holy basil Leaf extract 1 %	69.04	72.7	73.11	71.46	71.58	4.5	5.9	6.6	6.1	5.8
T_3 – Holy basil Leaf extract 3 %	70.91	73.21	73.68	69.71	71.88	5.9	6.4	7.2	6.1	6.4
T_4 – Holy basil Leaf extract 5 %	69.14	75.63	79.42	74.64	74.71	6.5	7.0	7.9	6.8	7.1
T_5 – Holy basil Leaf extract 10 %	72.49	76.17	75.61	64.33	72.15	5.0	6.8	6.3	5.7	6.0
Mean	69.80	73.85	74.93	69.62	72.05	5.2	6.4	6.8	6.0	6.1
T_0 – Unprimed seeds	65.17					3.0				
	T		D	T × D		T		D	T × D	
SEd	1.380		1.234	2.759		0.118		0.105	0.235	
CD (P=0.05)	2.788		2.494	5.577		0.238		0.213	0.476	

Table 2. Effect of holy basil leaf extract seed priming on germination percentage, root length and shoot length of Groundnut var. VRI 8

Priming agent (A)	Germination (%)					Root length (cm)					Shoot length (cm)				
	Soaking duration in h (D)					Soaking duration in h (D)					Soaking duration in h (D)				
	2	4	6	8	Mean	2	4	6	8	Mean	2	4	6	8	Mean
T_1 - Hydropriming	76 (60.71)	85 (67.55)	82 (64.97)	80 (63.52)	81 (64.19)	12.9	13.2	15	15.3	14.1	11.8	12.1	13.2	13.6	12.7
T_2 – Holy basil Leaf extract 1 %	78 (62.06)	81 (64.29)	85 (67.31)	79 (62.78)	81 (64.11)	14.7	15.2	16.6	16.9	15.9	12.9	13.1	14.1	14	13.5
T_3 – Holy basil Leaf extract 3 %	83 (65.76)	81 (64.19)	89 (71.15)	87 (69.24)	85 (67.59)	15.5	16.9	17.5	17.1	16.8	13.4	13.6	14.7	14.3	14.0
T_4 – Holy basil Leaf extract 5 %	85 (67.31)	88 (70.26)	94 (76.23)	91 (73.45)	90 (71.81)	16.9	17.4	18.2	17.8	17.6	14.1	14.2	15.2	14.8	14.6
T_5 – Holy basil Leaf extract 10 %	82 (64.97)	79 (62.78)	80 (63.52)	84 (66.75)	81 (64.50)	13.5	13.9	17.3	16.2	15.2	12.5	12.5	13.5	13.8	13.1
Mean	81 (64.16)	83 (65.82)	86 (68.63)	84 (67.15)	83 (66.44)	14.7	15.3	16.9	16.7	15.9	12.9	13.1	14.1	14.1	13.57
T_0 – Unprimed seeds	72 (58.05)					11.4					10.9				
	T			D	T × D	T		D		T × D	T		D		T × D
SEd	1.586			1.419	3.173	0.306		0.273		0.611	0.260		0.232		0.519
CD (P=0.05)	3.206			2.868	6.413	0.618		0.553		1.236	0.525		0.470		1.050

Table 3. Effect of holy basil leaf extract seed priming on dry matter production and vigour index of Groundnut var. VRI 8

Priming agent (A)	Dry matter production (g)					Vigour Index				
	Soaking duration in h (D)					Soaking duration in h (D)				
	2	4	6	8	Mean	2	4	6	8	Mean
T_1 - Hydropriming	2.09	2.20	2.78	2.53	2.40	1877	2151	2312	2312	2163
T_2 – Holy basil Leaf extract 1 %	2.43	2.28	2.98	2.90	2.65	2153	2292	2610	2441	2374
T_3 – Holy basil Leaf extract 3 %	2.81	2.55	3.37	3.12	2.96	2399	2471	2866	2732	2617
T_4 – Holy basil Leaf extract 5 %	2.93	3.15	3.82	3.54	3.36	2635	2781	3140	2967	2881
T_5 – Holy basil Leaf extract 10 %	2.73	2.90	3.10	2.95	2.92	2132	2086	2464	2520	2300
Mean	2.60	2.62	3.21	3.01	2.86	2239	2356	2678	2594	2467
T_0 – Unprimed seeds	1.95					1809				
	T		D		T × D	T		D		T × D
SEd	0.055		0.049		0.110	47.540		42.521		95.079
CD (P=0.05)	0.112		0.100		0.223	96.083		85.939		192.166

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RESEARCH ARTICLE

Identification of SSR Markers for Differentiating Some Traditional Paddy (*Oryza sativa L.*) Varieties in Cauvery Delta Region of Tamil Nadu

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ABSTRACT

Forty-four traditional landraces of Rice (*Oryza sativa* L.) collected from different parts of Cauvery delta regions of Tamil Nadu, India and the traditional rice seeds were raised in the Thenpathi village of tiruvarur district. Fresh young leaves were collected from each rice landrace cultivar for genomic DNA extraction using CTAB method. Ten SSR primers were selected for DNA amplification amoung 44 traditional vaieties 445 bands were produced. In which primer RM 6933 produced more bands of about 52. RM 481 produced unique di allilic band while amplified with kumsala. In Cluster analysis, dendogram was constructed using Jaccard similarity co efficient and suggested that high genetic diversity based on molecular allele distribution.

Keywords: Traditional rice, CTAB, SSR Primer, Dendrogram

INTRODUCTION

Rice is the most widely consumed staple food for over half of the world's human population. It belongs to the grass family Poaceae (2n = 12). There are 25 species of the genus *Oryza* and only two species, namely *O. sativa* and *O. glaberrima* are cultivated [1]. Rice is cultivated in more than 100 countries and produces more than 0.7 billion tonnes per annum with an area of about 0.158 billion hectares (0.470 billion tonnes of raw rice). Asia produces almost 0.640 billion tonnes of paddy, which contributes to 90% of the global needs. India has a rich and extensive variety of heritable rice. Approximately 425,500 accessions of rice varieties maintained in different gene banks across the globe

are possible genetic resources for crop improvement [2]. The farmer's variety is almost homogenous and is traditionally selected and cultivated by the farmers in their own field and is an improvement over the wild relatives and or land races [3]. The genetic purity of varieties is conventionally determined by the grow-out test (GOT), which is based on the assessment of phenotypical and floral characteristics called "descriptors" in plants grown to maturity. However, it is a time-consuming and resource-intensive exercise that is influenced by environmental factors and the results are frequently subjective [4]. In the process of seed production and multiplication, plant breeders, farmers, certification agencies, seed testing laboratories and seed industry should know the specific phenotypical traits of the genotype for identification at different growth stages of the crop. The distinctness, uniformity and stability (DUS) testing done by essential Phenotypical traits and by emplying biochemical markers for varietal identification is selective to ecological influence [5]. DNA fingerprinting is a useful tool for varietal protection to prove ownership or derivation of plant lines. Moreover, the analysis of genetic diversity and relationship between or within different species, population and individuals is a prerequisite towards effective utilization and protection of plant genetic resources[6]. Screening of traditional genotypes using SSR markers would create a valuable database for varietal identification, measure the extent of genotypic differences, genetic relationship and assist in broadening of genetic bases of the cultivars. The present study was thus aimed to evaluate the molecular variation among the traditional genotypes so that genetic relationship could be determined for utilization and further improvement of the existing lines.

MATERIALS AND METHODS

- **Experimental material**

The experiment was conducted with Forty-four traditional rice genotypes (Table. 1). The genotypes were collected from Centre for Indian Knowledge Systems, Sirkali, Cauvery Delta region of Tamil Nadu.

- **Experimental season and site**

The genotypes were raised in Randomized Block Design (RBD) with three replications with recommended spacing of 20 cm between rows and 15 cm between plants in 3m long rows. During the samba (August to January) season in Thenpathi Village (Latitude and longitude of 10°43'30.2"N 79°27'11.6"E) of Thiruvarur District, Tamil Nadu, India.

- **Molecular analysis**

DNA isolation : Fresh young leaves were collected from each Traditional rice cultivar for genomic DNA was extracted. Extraction of total genomic DNA was carried out by using the CTAB method [7] with some modifications.

Molecular Markers

A total of Ten SSR primers synthesized by Prr Biotech Innovations Pvt. Ltd. Hyderabad, were used for PCR amplification. The details of SSR primer used for PCR amplification are given in Table 2.

Amplification of genomic DNA using SSR primers through polymerase chain reaction (PCR)

The genomic DNA of the different rice genotypes isolated as described earlier were subjected to PCR amplification in thermal cycler the reaction volume of 15 µl containing 2 µl of genomic DNA 1X assay buffer, 200 mM of deoxy ribo nucleotides, 2 µM of $Mgcl_2$, 0.2 µM of primer, 1 unit of Tag DNA polymerase and 6.6 µl of sterile water. Annealing temperature was standardized for each primer and adopted for all the primers used in the study as identified by their specific Tm requirement (Table 3).

Cluster analysis

The scoring data in the form of binary values was used for the construction of dendrogram. The genetic associations between varieties were evaluated by calculating the Dice's similarity coefficient for pair wise comparisons based on the proportions of shared bands produced by the primers[8]. Similarity matrix was generated using the SIMQUAL

programme of NTSYS-pc software, version 2.02 [9]. The similarity coefficients were used for cluster analysis and dendrogram was constructed by the Unweighted pair-group method arithmetic mean (UPGMA)[10].

RESULTS AND DISCUSION

The results of the experiment including 44 traditional rice genotypes using ten molecular markers of Simple Sequence Repeat (SSR) are presented here under. Totally 445 bands were produced during the amplification process, all the ten primers showed polymorphism while they are amplified with the collected traditional rice genotypes (Fig. 1-10). While amplification process primer RM 6933 produced of about 52 bands which is recorded as the highest of these ten primers. RM 1812 produced 50 bands which is recorded as the second highest. Where as RM 249 produced the lowest band count of about 39 RM 316 produced 43 bands, RM 481 produced 45 bands, RM 858 produced 42 bands, RM 330 produced 45 bands, RM 5900 produced 46 bands, RM 7200 produced 42 bands and RM 8085 produced 41 bands. In the primer RM 249 **(**Fig. 1**)** the bands were produced in four different base pair. G1, G2, G4, G8 and G13 does not produced any bands while amplifying with RM 249 primer. Unique bands were produced in G3 and G5. In RM 316 (Fig. 2) the bands were produced in two different base pair. G1 does not produced any bands while amplifying with RM 316 primer. During the amplification process G18 alone produced di allele with RM 481(Fig. 3) primer. This primer showed different banding pattern in two different base pair. RM 848 produced two different banding patterns (Fig. 4). G18 and G20 does not produced any bands while amplified with RM 858 primers. RM 1812 (Fig. 5) produced of about 50 bands in two different base pair, where as some genotypes like G6, G8, G10, G11, G12 and G21 has produced di allele. During the amplification process RM 330 (Fig. 6) produced 45 identical bands in two base pair, whereas G2 has alone shown di allele. RM 5900 produced 46 bands in three different base pair (Fig. 7). G4 doesn't produce any band with RM 5900. G18 and G25 has produced di allele while amplified with primer RM 5900. RM 6933(Fig. 8) produced 52 bands in three different base pair. G1 and G7 produced di allele with this primer. Some genotypes like G2, G38, G40, G42 and G44 doesn't produce any bands with RM 6933 primer. RM 7200 (Fig. 9) produced 42 bands in two different base pair, while G20 and G33 doesn't produce any bands with this primer. RM 8085 (Fig. 10) produced 14 bands in three different base pairs, whereas G3, G43 and G44 does not produce any bands while amplified with RM 8085 primer.

CLUSTER ANALYSIS

Dentrogram of 44 traditional rice genotypes constructed from UPGMA cluster analysis using Jaccard's similarity coefficient based on data derived from 10 SSR markers. UPGMA was performed using Jaccard's similarity coefficient matrices calculated from 10 SSR markers to generate a dendogram for 44 Traditional rice genotypes. It ranged from 0 to 0.1 indicating the genetic diversity among the 44 varieties. The dendogram showed the grouping pattern of two main clusters all the genotypes G1- G43 were grouped in the first cluster and G44 alone in the cluster second cluster. Two sub cluster were grouped from the first main cluster, in this sub cluster 25 genotypes were grouped in a cluster and 18 genotypes in the other group. There is more formation of sub cluster due to high genetic diversity the cluster pattern were cleary showed in the Table 4 &5 and Fig 11. These clustering pattern shows that the traditional rice varieties cultivated in delta region of tamil nadu were genetically diversified especially vellaipoonkar is the traditional gentyoe which is more diversed from other rice varieties. According to the study Neelan samba and Salem samba were placed in the same cluster since they are genetically similar they fall in the same cluster. Poompalai and Puzhithi samba were also genetically similar hence they were placed in the same cluster. The same allel was amplified by RM 255 in traditional Basmati rice varieties, which was different to that in IR 36 and Azucana. They suggested to use RM 255 marker to differentiate traditional Basmati and non Basmati rice varieties [11]. RM 249 can be used to identify Arikiravi and Garudan samba but the formation of unique bands. RM 481 can be used to identify Kumsala by the formation of diallele. RM 848 can be used to identify some genotypes like Iraivanpodi, Kaliyan samba, Kamban samba, Karunguruvai, Karuppukowni and mullan Kaiama by the formation of diallele. RM 330 can be used to identify Ananthanoorsannaa by the formation of diallele. RM 5900 can be used to identify Kumasala and Panankattukudaivazhai by the formation of diallele. RM 6933 can be used to identify Anikomban and Kaivarai samba by the formation of diallele. Varietal identification by means of molecular analysis is most authenticated as

compared to all other method. The use of fluorescent labeled sample sequence length polymorphism for basmati rice adulterations [12]. In Del marker to distinguish basmati from other fragrant rice varieties[13]. A comparison of calibration method for quantification of basmati and non-basmati rice using microsatellite analysis was performed [14]. Many SSRs have been developed for rice to use genetic diversity studies . It was reported that more than 20000 SSR markers were mapped for genome-specific regions in rice . Many genetic diversity and characterization studies were done by using SSRs in rice [15]. The polymorphism detected among the genotypes will be helpful in selecting genetically diverse parents in the future breeding programme.

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Table 1. List of traditional rice varieties collected from delta regions of tamil nadu

Genotype Code	Name of the Variety	Genotype Code	Name of the Variety
G 1	Anaikomban	G 23	Ottadai
G 2	Ananthanoorsanna	G 24	Pachaiperumal
G 3	Arikiravi	G 25	Panakattukudaivazhai
G 4	Arubatham samba	G 26	Pisini
G 5	Garudan samba	G 27	Poompalai
G 6	Iraivapondi	G 28	Poovan samba
G 7	Kaivari samba	G 29	Puzhithi samba
G 8	Kaliyan samba	G 30	Rasagadam
G 9	Kallurundaikaar	G 31	Sadakar
G 10	Kamban samba	G 32	Salem samba
G 11	Karunguruvai	G 33	Samba
G 12	Karuppukowni	G 34	Samba mosanam
G 13	Kattuyanam	G 35	Sanna samba
G 14	Kitchili samba	G 36	Sengini
G 15	Kollikar	G 37	Sivapukownii
G 16	Kottara samba	G 38	Sivapukurivikar
G 17	Kullakaar	G 39	Sivapusirumani
G 18	Kumsala	G 40	Sooranku ruvai
G 19	Kuzhiadichan	G 41	Thanga samba
G 20	Mapillai samba	G 42	Thooyamalli
G 21	Mullan Kaiama	G 43	Veethivadangan
G 22	Neelan samba	G 44	Vellaipoonkar

Table 2. Details of ssr primers used for pcr amplification

S. No.	SSR-Primers	S. No.	SSR-Primers
1.	RM 3330	6.	RM 481
2.	RM 8085	7.	RM 585
3.	RM 249	8.	RM 1812
4.	RM 5900	9.	RM 6933
5	RM 316	10.	RM 7200

Table 3. Details of pcr amplification temperature and duriation

S. No	Stages	Temperature	Duriation
1.	Initial denaturation	94° C	5 minutes
2.	Denaturation (30 cycles)	94° C	30 seconds
3.	Annealing	50° C - 60° C	-
4.	Extension	72° C	30 seconds
5.	Final extension	72° C	8 minutes
6.	Cooling	14° C	-

Table. 4 Cluster 1 and Sub Cluster formation of 44 genotypes while amplified with 10 SSR marker.

<table>
<tr><td colspan="12">Sub cluster 1</td></tr>
<tr><td colspan="12">G32, G22, G21, G20, G30, G41, G37, G36, G43, G28, G29, G27, G39, G35, G34, G38, G17, G16, G15, G19, G42, G40, G33, G31, G18</td></tr>
<tr><td colspan="9">SC 1.1</td><td colspan="3">SC 1.2</td></tr>
<tr><td colspan="9">G32, G22, G21, G20, G30, G41, G37, G36, G43, G28, G29, G27, G39, G35, G34, G38, G17, G16, G15, G19, G42, G40, G33, G31,</td><td colspan="3" rowspan="5">G18</td></tr>
<tr><td colspan="5">SC 1.0.1</td><td colspan="4">SC 1.0.2</td></tr>
<tr><td colspan="5">G32, G22, G21, G20, G30, G41, G37, G36, G43, G28, G29, G27, G39, G35, G34, G38</td><td colspan="4">G17, G16, G15, G19, G42, G40, G33, G31</td></tr>
<tr><td colspan="5">SC 1.0.1.1</td><td colspan="4">SC 1.0.2.1</td></tr>
<tr><td colspan="5">G32, G22, G21, G20, G30, G41, G37, G36</td><td colspan="4">G43, G28, G29, G27, G39, G35, G34, G38</td></tr>
<tr><td colspan="3">SC 1.0.1.1.1</td><td colspan="2">SC 1.0.1.1.2</td><td colspan="4">SC 1.0.2.1.1</td><td colspan="3">SC1.0.2.1.2</td></tr>
<tr><td colspan="3">G32, G22, G21, G20, G30</td><td colspan="2">G41, G37, G36</td><td colspan="4">G43, G28, G29, G27, G39, G35, G34</td><td colspan="3">G38</td></tr>
<tr><td colspan="2">SC 1.0.1.2.1</td><td colspan="2">SC 1.0.1.2.2</td><td colspan="3">SC 1.0.1.1.2.1</td><td>SC1.0.1.1.2.2</td><td colspan="3">SC 1.0.2.2.1</td><td colspan="2">SC 1.0.2.2.2</td></tr>
<tr><td colspan="2">G32, G22, G21, G20</td><td colspan="2">G30</td><td colspan="3">G41, G37</td><td rowspan="4">G36</td><td colspan="3">G43, G28, G29, G27</td><td colspan="2">G39, G35, G34</td></tr>
<tr><td colspan="2">SC 1.0.1.3.1</td><td>SC 1.0.1.3.2</td><td rowspan="3"></td><td>SC 1.0.1.1.3.1</td><td>SC 1.0.1.1.3.2</td><td colspan="2">SC 1.0.2.3.1</td><td>SC 1.0.2.3.2</td><td>SC 1.0.2.2.2.1</td><td>SC 1.0.2.2.2.2</td><td>SC 1.0.2.2.2.3</td></tr>
<tr><td colspan="2">G32, G22, G21</td><td>G20</td><td colspan="2">G41</td><td>G37</td><td colspan="2">G43, G28</td><td>G 29, G27</td><td>G39</td><td>G35</td><td>G34</td></tr>
<tr><td>SC 1.0.1.4.1</td><td>SC 1.0.1.4.2</td><td rowspan="2"></td><td rowspan="2"></td><td rowspan="2"></td><td rowspan="2"></td><td>SC 1.0.2.4.1</td><td>SC 1.0.2.4.2</td><td rowspan="2"></td><td rowspan="2"></td><td rowspan="2"></td><td rowspan="2"></td></tr>
<tr><td>G32, G22</td><td>G 21</td><td>G43</td><td>G28</td></tr>
</table>

Table. 5 Cluster 2 and Sub Cluster formation of 44 genotypes while amplified with 10 SSR marker.

Sub Cluster 2																							
G10, G9, G14, G13, G25, G24, G23, G4, G1, G26, G3, G12, G11, G8, G7, G6, G5, G2																							
SC 2.1																		**SC 2.2**					
G10, G9, G14, G13, G25, G24, G23, G4, G1, G26, G3,																		G12, G11, G8, G7, G6, G5, G2					
SC 2.1.0													**SC 2.1.1**			**SC 2.2.0**							**SC2. 2.1**
G10, G9, G14, G13, G25, G24, G23, G4, G1													G26, G3,			G12, G11, G8, G7, G6, G5,							G2
SC 2.1.0.1													**SC 2.1.0 .2**	**SC 2.1.2 .1**	**SC 2.1.2 .2**	**SC 2.2.0.1**				**SC 2.2.0.2**			
G10, G9, G14, G13, G25, G24, G23, G4,													G1	G26	G3	G12, G11, G8, G7				G6, G5			
SC 2.1.0.2.1								**SC 2.1.0.2.2**								**SC 2.2.0.2.1**		**SC 2.2.0.2.2**		**SC 2.2.0. 2.1**		**SC 2.2.0. 2.2**	
G10, G9, G14, G13,								G25, G24, G23, G4,								G12, G11, G8,		G7		G6		G5	
SC 2.1.0.3.1				**SC 2.1.0. 3.2**				**SC 2.1.0.2.3.1**				**SC 2.1.0.2. 3.2**				**SC 2.2.0.3.1**		**SC 2.2.0. 3.2**					
G10, G9, G14,				G13				G25, G24, G23				G4,				G12, G11		G8					
SC 2.1.0.1.4.1			**SC 2.1.0.1. 4.2**					**SC 2.1.0.2.4.1**			**SC 2.1.0.2. 4.2**					**SC 2.2.0. 4.1**	**SC 2.2.0. 4.2**						
G10, G9			G 14					G25, G24			G23					G12	G11						
SC 2.1.0.1. 5.1	**SC 2.1.0.1. 5.2**							**S C 2.1.0.2. 5.1**	**SC 2.1.0.2. 5.2**														
	G9							G25	G24														

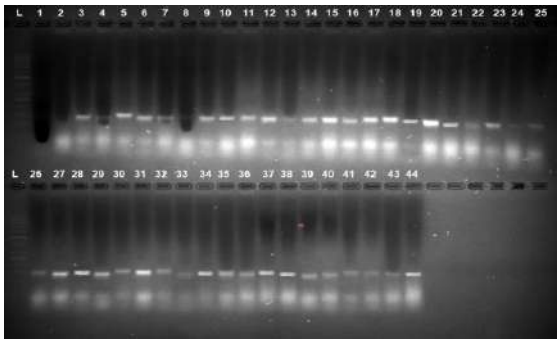

Fig. 1. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 249

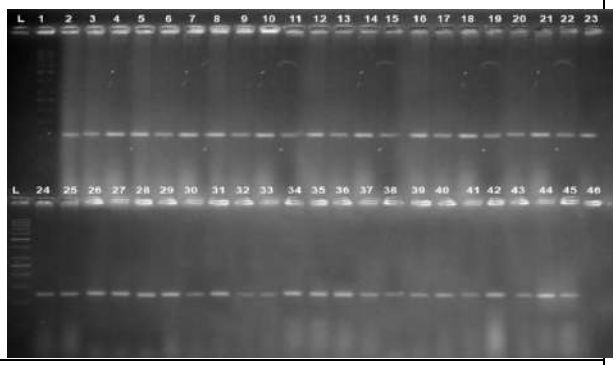

Fig. 2. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 316

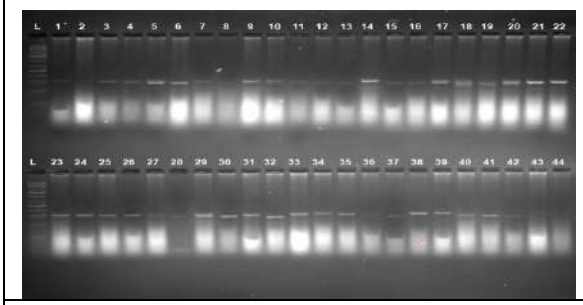

Fig. 3. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 481

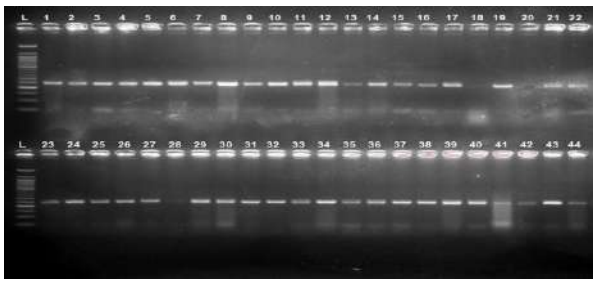

Fig. 4. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 858

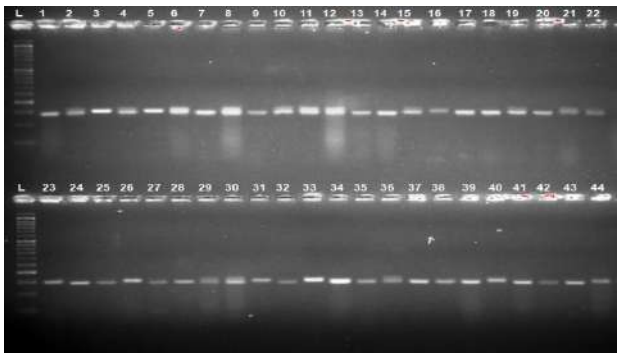

Fig. 5. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 1812

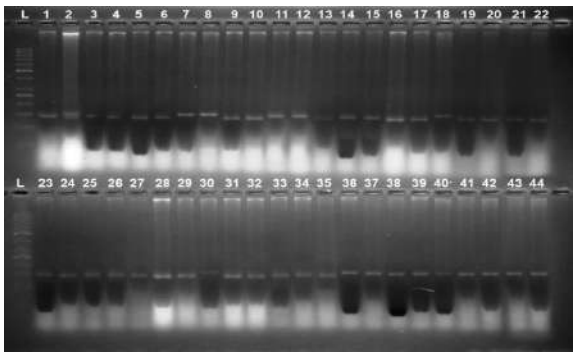

Fig. 6. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 3330

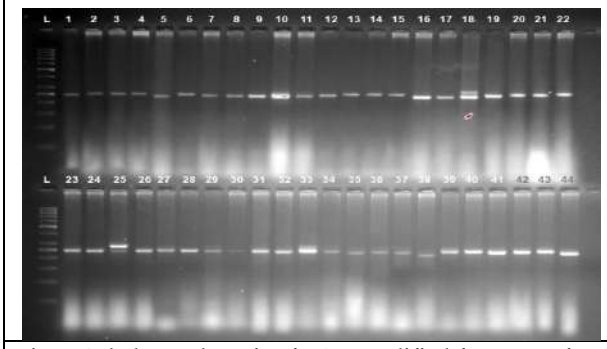

Fig 7. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 5900

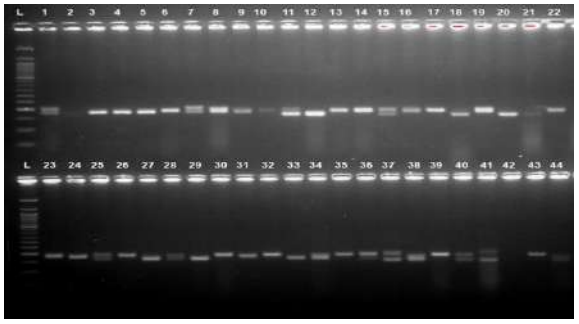

Fig 8. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 6933

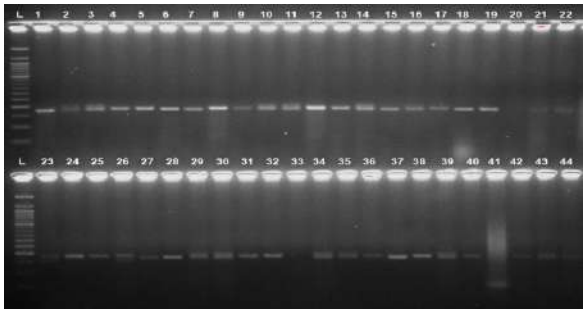

Fig 9. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 7200

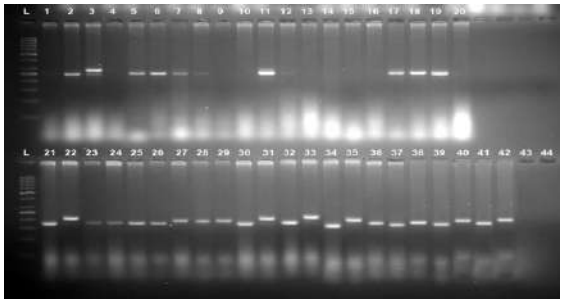

Fig 10. Gel electrophoresis of PCR amplified fragments for the SSR markers RM 8085

	L	**Ladder**	**L7**	**G7**	**L14**	**G14**	**L21**	**G21**	**L28**	**G28**	**L35**	**G35**	**L42**	**G42**
	L1	**G1**	**L8**	**G8**	**L15**	**G15**	**L22**	**G22**	**L29**	**G29**	**L36**	**G36**	**L43**	**G43**
	L2	**G2**	**L9**	**G9**	**L16**	**G16**	**L23**	**G23**	**L30**	**G30**	**L37**	**G37**	**L44**	**G44**
	L3	**G3**	**L10**	**G10**	**L17**	**G17**	**L24**	**G24**	**L31**	**G31**	**L38**	**G38**		
	L4	**G4**	**L11**	**G11**	**L18**	**G18**	**L25**	**G25**	**L32**	**G32**	**L39**	**G39**		
	L5	**G5**	**L12**	**G12**	**L19**	**G19**	**L26**	**G26**	**L33**	**G33**	**L40**	**G40**		
	L6	**G6**	**L13**	**G13**	**L20**	**G20**	**L27**	**G27**	**L34**	**G34**	**L41**	**G41**		

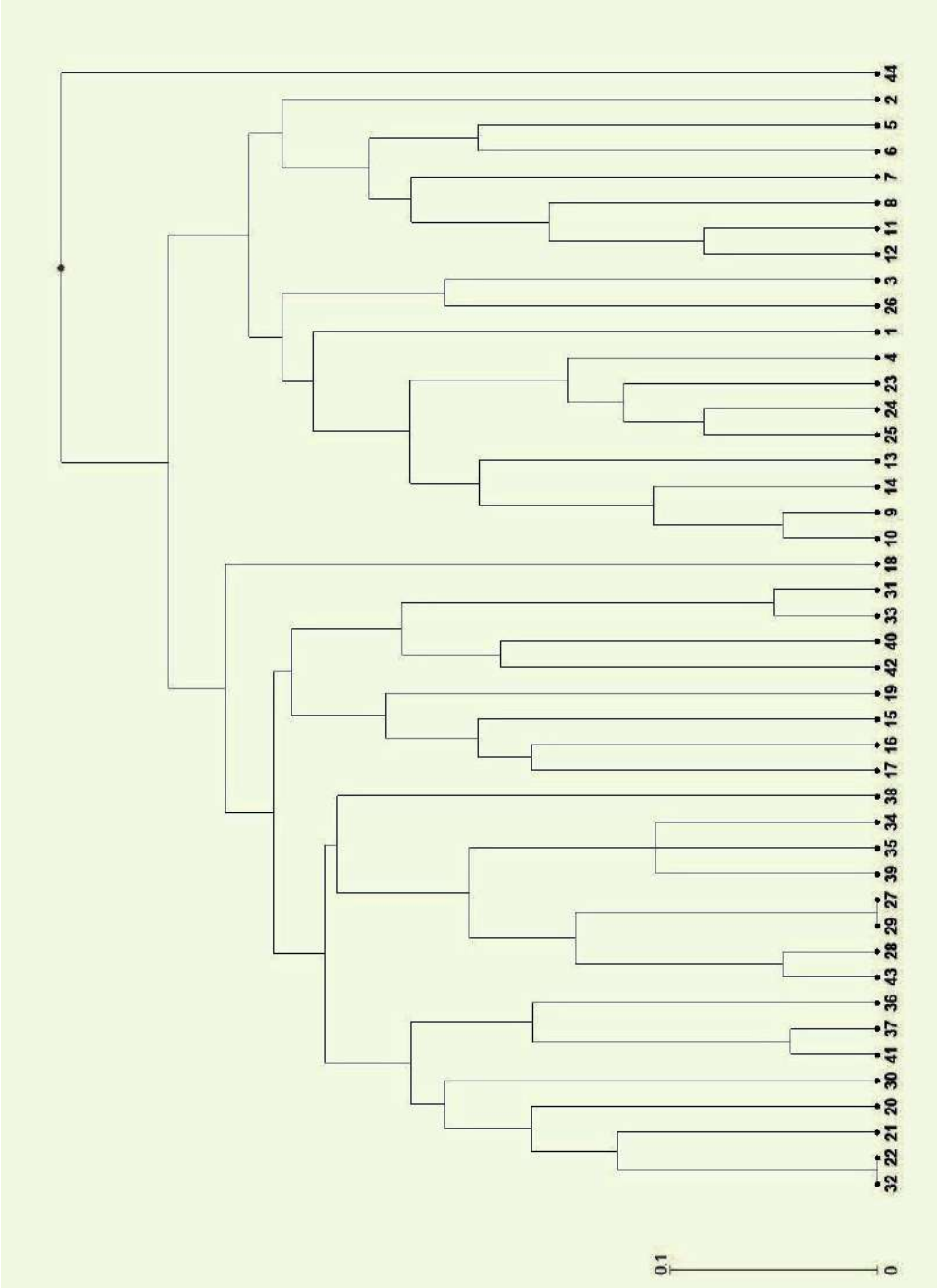

Fig 11. Dendogram UPGMA cluster analysis

REVIEW ARTICLE

Pharmacological Properties of North Indian Herbs of *Cichorium intybus* - Review

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ABSTRACT

All around the world, attention has recently shifted to plant studies. Chicory, also known as *Cichorium intybus,* is widely used medicinally to treat a variety of illnesses, from wounds to diabetes. utilizing chicory as a substitute for coffee has been known for many years. Despite having a long history of folkloric use, the pharmacological potential of several of its compounds has not been investigated. It is a perennial herb and reproduces through seeds and roots. Native to Europe, western Asia, and the central part of Russia, it was commonly farmed in ancient Europe. It is particularly significant as a levulose source. A plant that is grown in India is used as a tonic to treat diarrhea, spleen enlargement, fever, and vomiting. The wild form is regarded as emmenagogue, alexiteric, and tonic.

Keywords: *Cichorium intybus,* pharmacological potential, emmenagogue, alexiteric, and tonic

INTRODUCTION

The chicory plant commonly known as *Cichorium intybus* Linn is most likely a combination of numerous Greek and Latin words. In Latin Cichorium means "field," and both languages have a word for it called intybus [1]. The Greek equivalent refers to the morphology of the leaves and means "to cut." The Latin word tubes, which translates to "(a) tube," characterizes the stem's structure in turn. While the species name identifies the plant itself, the genus name refers to its habitat. The Greek word kichorion, which was frequently used in the writings of ancient physicians, was Latinized to get the name Cichorium. *Cichorium intybus* is a perennial plant that belongs to the family *Asteraceae* that has somewhat woody leaves and bright blue flowers most of the time, but rarely white or pink ones. The chicons or

roots are baked, pulverized, and used as a substitute for coffee. Inulin, extracted from chicory root, has been utilized as an artificial sweetener and dietary fiber source in the twenty-first century [2].For centuries, people all throughout the world have utilized the herbaceous plant Cichorium intybus, of the family Asteraceae, as feed for animals. Chicory's popularity is rapidly rising as a result of its many health, culinary, and nutritional benefits. The chicory extracted from roots is used as a substitute for coffee, animal feed, and pet food, while the leaves and blossoms are typically eaten as vegetables in salads. In order to enhance the flavor of alcoholic and non-alcoholic beverages, chicory extracts are occasionally added [3]. The insulin-rich tuberous roots of the plant can also be fermented to produce alcohol. Parts of the chicory plant are utilized as an ethnoveterinary treatment for physical maladies and diseases as well as preventative measures in both people and livestock in several regions. Due to the somewhat bitter flavour of fresh chicory roots, they are typically debittered by a certain process like boiling in water or citric acid solution and then being diced or milled before being utilized as feed, a functional food ingredient, or in coffee blends. Certain essential phytochemicals such as Inulin, a polysaccharide, flavonoids, coumarins, alkaloids, tannins, and volatile oil are identified in the roots of the chicory plant.[4] Flavonoids, tannins, and coumarins, which are secondary metabolites, found in chicory, have been documented to exhibit biological actions including antioxidant, anticancer, anti-inflammatory, antiparasitic, antibacterial, antifungal, antimalarial, antitumor, antidiabetic, anticarcinogenic, antimutagenic, anticoccidial, gastro-protective diuretic, immunogenic, and antihepatotoxic, which have a good impact on human and animal health [5].

Around 68 percent of the whole chemicals found in fresh chicory roots are made up of insulin, a polymer of fructose with - (2-1)-glycosidic-linkage [6]. Because it is prebiotic and low in calories and dietary fiber, insulin is a great alternative to sugar and a great addition to diabetic nutrition [7].With varying degrees of success, chicory has been utilized as a growth booster in poultry feed. However, a few studies have revealed that chicory has beneficial effects in various animal models, although the literature is lacking information regarding the mechanism by which chicory is employed as a *hepatoprotective* in the production of poultry [8]. The need for the study of chicory intybus is to understand the potential of this medicinal plant as a heat protectant to treat liver illnesses, assist in the replacement of antibiotics with chicory, and offer a promising future for usage as a herbal liver tonic in the chicken industry to reduce the cost of medications [9][10].

Synonyms

The chicory is commonly known as blue daisy, horseweed, blue dandelion, blue sailors, blue weed, bunk, coffee weed, cornflower, hendibeh, ragged sailors, succory, wild bachelor's buttons, and wild endive [11]

Biological Source

It is obtained from the blue-flowered *Cichorium intybus* perennial plant which belongs to the Family- *Asteraceae*.[12]

Geographical Source

Originally from Europe, western Asia, and central Russia, it was commonly grown throughout ancient Europe. root chicory, a plant primarily grown in northwest Europe, India, South Africa, and Chile, is used as a replacement for coffee or to extract inulin. Currently grown in the majority of temperate zones, which has escaped and established itself as a severe weed in many locations [13].

Chemical Constituents

Chicory contains many compounds from all parts, including roots, herbs, flowers, and leaves. Chicory acid has been determined to be the main component of methanolic chicory extract [14]. *Terpenoids* are trace components of plants but are dominated by aliphatic chemicals and their derivatives. The aerial part of chicory had the highest concentration of ascorbic acid (mg / 100 g), followed by seeds (23.85) and roots (43.11). (9.34). According to Helaly and Abdullah, ascorbic acid levels in chicory native species in eight different parts of Egypt ranged from 15.33 to 33.92 mg / 100 g. Starch content (mg / g) was highest in the above-ground part of the chicory (17.28), followed by seeds (5.57) and roots (3.22), followed by tannin content (mg / g) in the above-ground part of the chicory. rice field. Highest (614), followed by roots (2.21) and seeds [15]. Fresh chicory root contains 68% insulin, 14% sucrose, 5%

cellulose, 6% protein, 4% ash, and 3% other compounds. Dried chicory root contains 98% insulin and 2% other compounds [16]. In addition to 2-acetylpyrrole, furfural, *phenylacetaldehyde, phenylacetic acid,* vanillin, *pyrazine, benzothiazole, aldehydes*, aromatic hydrocarbons, furan, phenol, organic acids, trace amounts of indole alkaloids (β) in roasted chicory roots. -Carboline) is also included), Harmane and still Harmane. The roots of the common chicory plant contain flavonoids, tannins, volatile oils, and other essential elements that are extracted by removing insoluble particles and followed by filtration and centrifugation.[17]. More than 76% of the total fatty acid profile, including monounsaturated oleic acid (18: 1n-9), stearic acid (18: 0), and palmitic acid, is found in seed-rich, soothing oils. It is an excellent source of both saturated and unsaturated fatty acids [18]. Fructose is a type of carbohydrate, long chain, composed of 22-60 molecules and has a glucose molecule at the terminus, which is abundant in chicory (up to 94%) [19]. Chicory leaves contain phenolic compounds, inulin, vitamins A, B1, B2, C, Ca, K, Mg, Na, Cu, Mn, and other essential elements. *Cichorium intybus* seed extract/fraction, total flavonoid levels ranged from 43.3 to 150 CE mg / 100 g dry plant material [20]. Saccharide, methoxy coumarin, chicory, flavonoids, essential oils, and anthocyanins are found in chicory flowers that contribute to the blue colors of the perianth [21]. Seeds are known to contain high levels of reducing sugars, while leaves are known to contain relatively high levels of total and non-reducing sugars. We also found that the levels of free amino acids and water-soluble proteins in the leaves were high.

With the exception of the salt-soluble protein content, the content of all components examined in the roots was reduced [22]. Steam distillation and liquid-liquid extraction were used in a two-step extraction process to obtain volatile chemicals. The distillate was extracted using pentane. Then the organic layer is separated, dried over anhydrous sodium sulfate, concentrated to 0.5 ml under reduced pressure, and then removed. The concentrated extract was pale yellow and had a strong odor. A combination of GC-FID and GC-MS was used to isolate and identify various oil components. The 20 ingredients found in the aerial part of the herb were found inoil after analysis. Carvacrol 50.1%, thymol 13.3%, cinnamaldehyde 12.4%, Camphor 4.4%, carvone 4.1%, linalool 3.9%, and -terpineol 3.9%made up the majority of the mixture (2.1 percent) [23].Flowers contain a variety of sugars, cicorin, and coumarin derivatives such as umbelliferon, esculin, which is known as esculetin 7-O-glucoside and scopoletin, silicic acid, taraxosterol, valeric acid, flavonoids (hyperoside), etheric oils, and anthocyanins, the latter of which is responsible for the perianth's blue hue. Gum, choline, phytosterols, mucus, copper, latex, lipids, proteins, P and K vitamins, amino acids, -sitosterol, malic acid, oxalic acid, shikimic acid, quinic acid, succinic acid, tannins, saponins, flavonoids, terpenoids, cardiac glycosides, and other substances found in the plant [24] & [25].

Pharmacological Activities

The plant *Cichorium intybus* is understudied in terms of pharmacology and phytochemistry. Most the pharmacological research on this plant show the testing of mainly alcoholic and/or aqueous extracts. Additionally, intybus (hairy root cultures) have been linked to DDT *phytoremediation*. The seeds of *Cichorium intybus* are used in the Unani and ayurvedic medicine system in the treatment of liver, constipation, heart, and other condition.

Anti-Inflammatory Effect

Chicory is treated with topical as compresses to treat a variety of skin conditions, including dermatitis, mucous membrane inflammation, ulcers, wounds, and trauma. Prostaglandin E2 (PGE2) synthesis in human colon cancer HT29 cells also treated with the pro-inflammatory compound TNF-alpha was significantly inhibited by ethyl acetate chicory root extract [25].

Anti-cancer effect

Chicory root ethanolic extract inhibited the growth of Ehrlich ascites carcinoma in mice, according to research 500 mg/kg of 8 doses was introduced intraperitoneally, and was found to observe a 70% increased life span in mice.[26]. Magnolialide, a 1-hydroxyeudesmanolide found in *Cichorium intybus* roots, inhibited many tumour cell lines and caused the differentiation of human leukemia HL-60 and U-937 cells into monocyte- or macrophage-like cells [27]. On amelanotic melanoma C32 cell lines, the aqueous-alcoholic macerate of *Cichorium intybus* leaves had an antiproliferative effect. Human prostate cancer PC-3 cells, human breast cancer T47D cells, and colon cancer RKO cells were used as test subjects for *Cichorium intybus* aqueous extracts' anticancer abilities. In all three cancer cell lines,

Cichorium intybus extract showed a slight reduction in cell proliferation. *Cichorium intybus* (seeds) showed a 5–24% suppression in cell viability at a concentration of 1–10% for 24 hours. Cichoriin, anthocyanins, lactucin, and lactucopicrin were only a few of the photosensitive substances found in chicory [28].

Anti-Microbial Effect

The antibacterial effect of *Cichorium intybus*, Gram-positive and Gram-negative bacteria were both resistant to the antibacterial activities of the chicory root ethyl acetate extract. Conversely, the chicory hexane extract did not exhibit this. Aspergillus Niger and Saccharomyces cerevisiae were both resistant to the antifungal effects of the chicory root ethyl acetate extract [29].

Anti-Allergic Effect

Both in vitro and in vivo, the extract of Chicory intybus blocked the cell-mediated allergic reactions. This extract stopped the systemic dose-dependent anaphylaxis response in mice. It reduced passive cutaneous anaphylactic reaction caused by anti-dinitrophenyl IgE in rats. Other Histamine production from rat peritoneal mast cells and plasma histamine levels are both indicators of an allergic reaction. considerably diminished, although the levels of CAMP increased [30].

Anti-Diabetic Effect

An anti-diabetic effect of chicory has been found. The hypoglycemic and hypolipidemic effects of *Cichorium intybus* have been used traditionally in diabetes mellitus. Characteristics of the plant's whole ethanol extract were investigated. Male Sprague-Dawley rats were given streptozotocin intraperitoneally to cause diabetes. The extracted ethanol when administered at a dose of 125mg per kg has resulted in a decreased serum glucose level. Both early-stage and late-stage diabetic rats received therapy with chicory extract to stop weight loss [31].

Hepatoprotective Effect

The Wistar strain of Albino rats was tested for their anti-hepatotoxic properties against carbon tetrachloride-induced hepatic damage using *Cichorium intybus* natural root and root callus extracts. The extracts obtained from root and callus tissue along with the carbon tetrachloride showed a significant decrease in the elevated level of bilirubin and serum enzymes. Rats treated with natural root and root callus extracts and carbon tetrachloride showed an increase in the lowered levels of albumin and proteins seen in rats following carbon tetrachloride treatment. Histopathological evaluation of the liver portion supported these biochemical data [32].

CONCLUSION

Cichorium intybus has been known for its medicinal purposes by traditional and other rural communities as a primary healthcare practice. The plant is well-known for its roots that is used as a coffee substitute and are widely used to treat a variety of diseases. This plant has a rich history of use in folklore as indicated by its different folk names. The current review focuses on the medicinal importance of the plant both in Ayurveda and modern science. The phytochemical components of chicory intybus were found to have hepatoprotective, anti-diabetic, anti-cancerous, anti-inflammatory, analgesic activity, cardiovascular activity, antioxidant property, antimicrobial, anthelmintic, antimalarial, antiallergic, and gastroprotective activities. There are several phytochemicals present in the C. intybus plant that are yet to be explored in the field of development and need more research and clinical studies to increase their efficiency and importance in modern sciences.

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Figure 1. ***Cichorium intybus*** **perennial plant**

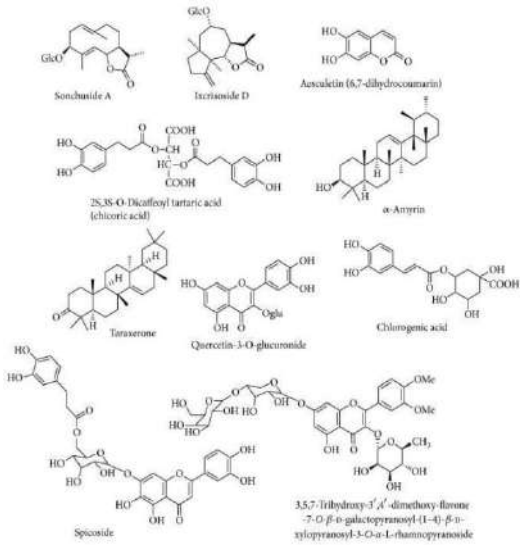

Figure 2. Chemical Constituents of ***Cichorium intybus***

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RESEARCH ARTICLE

Coping Strategies as a Predictor of Posttraumatic Growth among Border Residents of Jammu Division

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ABSTRACT

Although the positive changes after experiencing trauma have been well established, the role of coping strategies in it's development has not been properly studied. The present study aims to examine the predictive strength of coping strategies on the posttraumatic growth among border residents of Jammu. The sample consisted of 200 adults aged 35 years and above. The brief COPE scale and Posttraumatic growth inventory were used. Data was analysed using Pearson correlation and regression analysis. The results show that coping strategies were significantly correlated and predicted the posttraumatic growth in people who had experienced traumatic events. People who used adaptive coping strategies experienced high level of posttraumatic growth and the use of maladaptive coping strategies reduced the positive change in the individual after a traumatic event. This study further recommends that a culturally adapted psychological intervention program be developed to enhance he posttraumatic growth among the people living in border areas.

Keywords: coping strategies, brief COPE, posttraumatic growth, border residents.

INTRODUCTION

A conflict zone can be defined as one where armed forces are present and engaged in acts of warfare [1]. People living in conflict zones often suffer adverse mental health consequences. There is a high probability of them experiencing traumatic events like shelling and cross-border firing that might lead to mental health problems like anxiety, depression and post traumatic stress disorder [2]. The international border in Jammu division is known as the 'working boundary' after the Kashmir insurgency in 1989. In January 2015, there were a total of 253 incidents of cross-border firing along the international border. These incidents left 16 dead, 72 injured while 72 houses were

damaged. Around 1238 people were temporarily shifted to relief camps and around 2200 shifted to their relatives during that year. In the year 2016-17, a total of 187 people lost their lives or were gravely injured in the cross-border shelling. Around 121 houses were gutted in Jammu region and many schools and mosques were damaged in areas like Bandipora, Baramulla and Kupwara [3]. People residing in border areas have faced many hardships like destruction of property which not only leaves physical but also psychological wounds. Trauma is referred to the event in which an individual is exposed to the actual or threatened death or a serious injury. The exposure could be direct or indirect, like learning about a friend or a relative exposed to the trauma [4]. Macksoud and Aber [5] studied the effect of war related trauma and the relationship between traumatic incidents and mental health issues. Their research revealed that such events were strongly related to anxiety and PTSD.

Previous research has revealed that exposure to war like situations and constant anxiety were the main reasons for the mental health problems like PTSD and depression [6,7].In spite of aforementioned negative results of such events, recent findings suggest that some trauma victims show positive changes in their lives. Tedeschi and Calhoun [8] defined it as the Posttraumatic growth, a positive psychological change that follows a struggle with highly stressful life event. The Functional Descriptive Model says that growth occurs as a result of the endeavours made to reconcile with the event by reinterpreting it's meaning [9]. All the trauma victims may not experience this growth [10]. Posttraumatic growth is just the opposite of posttraumatic stress disorder, where only pain and anxiety is experienced by the victim [11]. Posttraumatic growth has been studied on events such as flood, earthquake, death, cancer, etc. [12]. The positive change in trauma victims are reflected in different areas of life such as increased personal strength, better relationship with family and friends, change in philosophy, spiritual changes , etc. [8]. Coping is a stabilizing factor that assists an individual in adapting to a stressful event in their life [13]. Methods of coping can be cognitive, behavioural, control, escape, social or solitary [14].

The concept of coping is defined as a person's response to a stressful situation with an aim to adapt psychologically to it [15]. It involves elements such as efforts to modify the problem at hand and creating solutions to the problem by evaluating it's merits and demerits [16]. Coping strategies can be divided into adaptive and maladaptive coping [17]. Coping strategies are categorized into three main divisions such as Emotion focused coping (use of positive reframing, acceptance, emotional support and humour), Problem focused coping (planning, use of instrumental support and active coping) and Dysfunctional coping (denial, substance abuse, venting, behavioural disengagement and self-blame) [18]. Many studies suggested that coping strategies could predict the prevalence of posttraumatic growth in cancer survivors, victims of flood and war etc. [19,10,20-22]. There were scarcely any such studies on the people living near hostile borders and most were held in western context. The present study focuses on contributing to the literature on the predictability of posttraumatic growth by coping strategies used by border residents. The objective of this study is to serve as a preliminary study in the research attempts to develop and enhance coping strategies and posttraumatic growth among people living near the hostile borders.

HYPOTHESES

H1: Coping strategies will have significant relationship with posttraumatic growth among border residents.
H2: Coping strategies will significantly predict posttraumatic growth among border residents.

MATERIALS AND METHODS

Sample

The study was conducted on a sample of 200 adults above the age of 35 years residing near the international border of Jammu division. The sample was drawn using the purposive sampling technique from the villages affected by firing and shelling in Samba, Ranbir Singh Pura and Akhnoor areas. The average age of the sample is 38.12 years.

Research design

The present study employed correlational research design in order to evaluate the relationship between coping strategies and posttraumatic growth.

Instruments used

Brief Coping Operations to Problems Experienced (Brief COPE)

It consists of a 28 item questionnaire created by Carver [23] that measures the coping styles of an individual. It rates the responses on a 4-point likert scale, where two items each form the following 14 subscales: active coping, planning, positive reframing, acceptance, humour, turning to religion, using emotional support, using instrumental support, self-distraction, denial, venting, substance abuse, behavioural disengagement, and self-blame. A high score indicates increased use of the respective coping strategy and vice versa. The test-retest reliability of the scale ranges from 0.50 to 0.90.

Posttraumatic Growth Inventory (PTGI)

Designed by Tedeschi and Calhoun [8], it is the most frequently used scale for measuring the positive changes resulting from a traumatic event. It consists of 21 items further subdivided into five domains: new possibilities, relating to others, personal strength, appreciation of life and spiritual change. The answers are scored a 6-point likert scale ranging from 0 (I didn't experience this change to a very great degree as a result of my crisis) to 5 (I experienced this change to a very great degree as a result of my crisis). The scores range from 1-105. A higher score on the PTGI indicates a greater degree of posttraumatic growth. The PTGI has been proved to have high construct validity and test-retest reliability of 0.71.

Procedure

The data was collected from the residents of the villages located near the international border in Jammu division. A total of 200 people participated in the study. With their informed consent, they were requested to fill up the questionnaires and were assured about the confidentiality and anonymity of data.

RESULTS

The data set met the assumption of multiple regression analysis and the normality of the data was examined using the Durbin Watson criteria. Table 1 shows that there is significant positive correlations between posttraumatic growth and emotion focused coping ($r=.75$, $p<.01$) and problem focused coping ($r=.76$, $p<.01$). As hypothesised, emotion focused coping and problem focused coping show significant positive correlation with posttraumatic growth and these results are consistent with the previous studies [10,21]. The correlation between posttraumatic growth and dysfunctional coping is significantly negative ($r=-.81$, $p<.01$). It justifies the second hypothesis that dysfunctional coping has a negative correlation with posttraumatic growth [24]. Table 2 shows the impact of each coping strategy on the posttraumatic growth individually. The findings revealed that EFC (B=.67) and PFC (B=.62) positively predicted the posttraumatic growth. While, the DFC (B=-.69) negatively predicted the posttraumatic growth. The contribution of each coping strategy was analysed. According to the results, all three coping strategies had a predictive effect on posttraumatic growth. In short, an increase in problem focused and emotion focused coping caused an increase in posttraumatic growth, while an increase in dysfunctional coping lead to a decrease in posttraumatic growth. The results of this study are consistent with the study conducted by Patri and Pietrantoni [25]. Individuals showing high level of emotion focused coping demonstrated high level of control on their emotions and feelings that result from the stress or trauma [26]. Whereas, problem focused coping strategies appear to counter the trauma by actively managing the stressful situations and alter the troubled person's environment to eliminate the source of the stress [27].

DISCUSSION

This study was meant to analyse the influence of coping strategies on posttraumatic growth among the people living near the international border in Jammu division of J&K. It was observed that individuals that adopted the emotion focused and problem focused coping had a better rate of positive changes in their lives after a stressful event. The first hypothesis of this study was to examine whether there exists a relationship between coping strategies and posttraumatic growth. And, the second hypothesis was to find out if coping strategies can predict posttraumatic growth. The results confirmed both of these hypotheses. It was observed that individuals who practiced adaptive coping strategies such as emotion and problem focused coping demonstrated higher positive changes in their life after suffering a stressful event. Whereas, the individuals who adopted maladaptive coping strategies like dysfunctional coping demonstrated no such positive changes in their lives. These results validate the previous studies among people affected by HIV, cancer and earthquake survivors (28). As the participants of this study belonged to one of the most hostile borders in the world (29), these results confirm the need to address the lack of mental health facilities provided to the victims of cross border firing or shelling. From a pure clinical perspective, this study calls the attention of the authorities and the mental health professionals to work out a culturally appropriate intervention program to help the people living in these areas enhance their coping strategies which will protect them from suffering negative consequences of the trauma.

CONCLUSION

Strengthening the coping strategies would be an advancing step in achieving the posttraumatic growth and limiting the negative effects of the stressful events. Greater involvement of grassroots level mental health workers and volunteers at primary and community health centres would act as a buffer against the negative consequences of the trauma.

RECOMMENDATIONS

In border areas where cross-border firings are a regular occurrence, it is important to raise awareness about the long term affects of the psychological trauma. There must be awareness programs in schools and community centres about how to enhance the adaptive coping strategies. It will help in creating a psychologically healthier community and society as a whole.

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Table1: Correlation between EFC, PFC, DFC and PTG

Variables	n	M	SD	1	2	3	4
PTG	200	44.17	13.25	-			
EFC	200	17.16	6.67	.75**	-		
PFC	200	12.89	5.13	.76**	.135**	-	
DFC	200	28.56	5.13	-.81**	-.076	-.039**	-

Note: PTG= Posttraumatic Growth; EFC= Emotion Focused Coping, PFC= Problem Focused Coping, DFC= Dysfunctional Coping

**: Correlation is significant at the 0.01 level.

Table2: Regression model: Coping strategies on posttraumatic growth

			Model Summary		
Model	R	R^2	Adjusted R^2	Standard Error of the Estimate	Sig.
1	.898[a]	.807	.803	5.90255	.001
a. Predictors: (Constant) EFC, PFC and DFC					
			ANOVA[a]		
Model		Sum of Squares	Df	Mean Square F	Sig.
1	Regression	18661.767	3	6220.589 178.547	.001b
	Residual	4459.529	128	34.840	
	Total	23121.295	131		
a. Predictors: (Constant), EFC, PFC and DFC					
b. Dependent variable: Posttraumatic growth					

Coefficients[a]					
Model 1	Unstandardized Coefficients	Standardized Coefficients			95%Confidence Interval
	B SE	Beta	t	Sig.	
(Constant)	45.422 3.956		11.481	.000	[75.15, 100.50]
EFC	.678 .108	.339	6.266	.006	[10.539, 15.877]
PFC	.624 .154	.241	4.502	.010	[-.290, .130]
DFC	-.693 .080	-.455	-8.636	.003	[-.471, -.299]
a. Dependent variable: Posttraumatic Growth					

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RESEARCH ARTICLE

Effect of Sulphur Fertilization and Foliar Nutrition on Growth Attributes of Blackgram cv ADT-3

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ABSTRACT

Field investigation was carried out to study the effect of sulphur fertilization and foliar nutrition on growth attributes of blackgram *cv* ADT-3 at the Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar. The field experiment was laid out in split plot design with 3 replications. The main plot constitutes RDF alone (M_1), RDF + 15 kg S ha^{-1} (M_2), and RDF + 20 kg S ha^{-1} (M_3). In sub plots, foliar nutrition practices *viz*., control (S_1), two per cent DAP foliar spray on 25 and 45 DAS (S_2), 0.5 per cent MAP foliar spray on 25 and 45 DAS (S_3), two per cent urea foliar spray on 25 and 45 DAS (S_4), two per cent water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS (S_5) and 0.5 per cent chelated micronutrient mixture foliar spray on 25 and 45 DAS (S_6). The result indicated that RDF + 20 kg S ha^{-1} combined with 0.5 per cent chelated micronutrient mixture foliar spray on 25 and 45 DAS (M_3S_6) recorded highest values of above growth components such as Plant Height, Leaf Area Index (LAI), Number of branches $plant^{-1}$, Dry matter production (DMP), Root length and Root nodule $plant^{-1}$ of blackgram *cv* ADT-3.

Keywords: Blackgram, Sulphur, Growth attributes, Chelated Micronutrient

INTRODUCTION

Pulses are the important sources of proteins, vitamins and minerals for the predominantly vegetarian population and are popularly known as “Poor man’s meat” and “rich man’s vegetable”. The heavy leaf drop

increases the organic matter in soil and several pulses can also fix large amount of nitrogen through symbiosis and thus minimize the dependency on chemical fertilizers. Thus pulses play a vital role in providing protein rich food to human beings and in sustaining both soil health and crop production on long-term basis. India is the largest producer and consumer of pulses in the world accounting for 35.2 per cent of world area and 30 per cent of world's production. With the inception of five year plans, India has achieved considerable progress in the production of other crops but the production and productivity of pulses remained more or less the same. As a result, the per capita availability of pulses in India has declined from 61 grams per day in 1951-56 to less than 40 grams in recent years as against Indian Council of Medical Research (ICMR) recommendation of 65 g per day. This shortfall has serious nutritional implications. Thus there is an urgent need to increase the production of pulses to meet the requirement, by manipulating the production technologies appropriately. Sulphur deficiencies in India are widespread and scattered. Deficiency of sulphur in Indian soils is on increase due to intensification of agriculture with high yielding varieties and multiple cropping coupled with the use of high analysis sulphur free fertilizers along with the restricted or no use of organic manures leading to depletion of the soil sulphur reserve. Crops generally absorb sulphur and phosphorus in similar amounts. On an average, the sulphur absorbed per tonnes of grain production is 8 kilograms in pulses, and 12 kilograms in oilseeds. Soils, which are deficient in sulphur, cannot provide adequate sulphur to meet crop demand resulting in sulphur deficient crops and sub-optimal yields [1].

Fertilizer is a vital input in agriculture to boost the crop yields. Among the methods of ferti1izer application, foliar nutrition is recognized as an important method as it facilitates easy and rapid utilization of nutrients. Foliar nutrition is a simple and cheaper technology which ensures the supply of nutrients to the crops directly where they are needed without spending energy for their transport, application and without any losses in transit. Plants deficient in micronutrients may become susceptible to diseases and abiotic stresses. The enhancement effect of foliar application of might be attributed to the favorable influence of these nutrients on metabolism and biological activity and its stimulation effect on photosynthetic pigments and enzymes activity which in turn encourage vegetative growth of plants [2]. Considering the above facts, a field experiment was conducted to study the effect of sulphur fertilization and foliar nutrition on growth attributes of blackgram ADT-3.

MATERIALS AND METHODS

The present Field experiment was carried out at Field number 9E of Experimental Farm, Department of Agronomy, Annamalai University, Annamalai Nagar. The Experimental Farm is geographically situated at 11° 24' North latitude and 79° 44' East Longitude and with an altitude of + 5.79 m above mean sea level. A popular black gram variety ADT-3 was raised with following treatment schedule by adopting Split plot Design with three replications under irrigated condition. The plot size was 4×3 m^2. The crop was raised with the spacing of 30 × 15 cm and recommended package of practices for blackgram were followed.

Main Treatments: sulphur application

M_1 – RDF alone (no Sulphur)
M_2 – RDF+ soil application of sulphur @ 15 $kgha^{-1}$
M_3 – RDF+ soil application of sulphur @ 20kg ha^{-1}

Sub Treatments: Foliar Spray

S_1 – Control (No Spray)
S_2 – Foliar spray of 2% DAP at 25 and 45 DAS.
S_3 – Foliar spray of 0.5 per cent MAP at 25 and 45 DAS.
S_4 – Foliar spray of 2% urea at25 and 45 DAS.
S_5 – Foliar spray of 2 % water soluble fertilizer (19:19:19) at 25 & 45 DAS.
S_6 – Foliar spray of 0.5 % chelated micronutrient mixture at 25 & 45 DAS.

The recommended dose of inorganic fertilizer nutrients *viz.,* nitrogen @ 25 kg ha^{-1} through urea, phosphorus @ 50 kg ha^{-1} through single superphosphate and potash @ 25 kg ha^{-1} through muriate of potash were applied to all the plots. As per the treatment schedule sulphur was applied at the rate of 15kg ha^{-1} and 20kg ha^{-1} through gypsum. For foliar spray application the required quantities of various foliar spray fluids were calculated plot wise and the quantity of spray fluid required per plot was determined and sprayed. The DAP solution was prepared on the previous day night by dissolving 10 Kg of DAP in 10 liters of water and allowed to settle overnight and it was filtered using a muslin cloth and the supernatant solution was taken for spraying after diluting with remaining quality of water (490 litres). The 0.5 per cent MAP was prepared by dissolving 5 grams of MAP in 1 liter of water. Stir or shake the mixture until all the contents is dissolved The 2per cent urea was prepared by dissolving 20 grams of urea in 1 liter of water. The 2 per cent water soluble fertilizer solution is prepared by dissolving 20 grams of water soluble 19:19:19 in 1 liter of water. The 0.5per cent chelated micronutrient is prepared by dissolving 5 grams of chelated micronutrient in 1 liter of water. The above foliar nutrients were sprayed using knap sack sprayer at 25 and 45 DAS. Proper irrigation was done at critical stages of flowering and pod formation and five plants from each plot were chosen by simple random sampling method and tagged. These tagged plants were used for recording all crop growth attributes such as Plant Height, Leaf Area Index (LAI), Number of branches plant $^{-1}$, Dry matter production (DMP), Root length and Root nodule plant^{-1}were observations at different stages of crop growth. The mean values of biometric observations were recorded and statistically analyzed as per the procedure outlined by [3].

RESULT AND DISCUSSION

Blackgram is an important source of dietary protein and energetic crop. Among the various factors affecting growth and yield of blackgram, nutrient management plays a vital role. The increase in plant height observed in the experiment may be due to the favorable effect of sulphuron. N metabolism and consequently on the vegetative growth of black gram plant. Similar finding was reported by [4]. From the experimental results, it is evident that the values of blackgram growth components *viz.,* plant height, Leaf Area Index and DMP at varied stages of crop growth were significantly high in (M_3). The treatment (M_3) recorded highest plant height of (36.13, 36.36) and (43.25, 45.36) cm followed by (M_2) (33.89, 25.62) and (40.82, 25.35) cm at flowering and harvest stages in black gram respectively. This was ascribed due to the increasing levels of sulphur could have increased the ferridoxin content which is responsible for nodulation activity. Ferridoxin are rich in sulphur and contain Fe-S clusters which play vital role in N_2 fixation. These nodulation encourage the activity of rhizhosphere region increases the nutrient retention in root zone which in turn increased the nutrient absorption and translocation from assimilate to shoot encourage the plant height of blackgram.In the present investigation higher leaf area index also was observed with application of RDF + 20 kg S ha^{-1} (M_3). This was ascribed due to the sulphur application which leads to higher absorption and translocation of nutrients assimilates to shoot thereby increased the number of leaves per plant in blackgram. This result was corroborating with the findings, who reported that among the sulphur levels, application of sulphur @ 20 kg ha^{-1} significantly increased the number of leaves per plant in blackgram as compared to no sulphur application [5].

The progressive dry matter accumulation has been recorded in flowering and harvest stages of the crop of 2039.16 and 2434.97 kg ha^{-1} respectively. Dry matter production increased with increased level of Sulphur at 20 kgs ha^{-1}. The effects on increased synthesis of amino acid and fatty acid with the soil application of Sulphur must have increased the dry matter accumulation [6]. This significant influence of sulphur application on increasing the growth might be attributed to its role in chlorophyll synthesis. The findings lend support to the present results [7] and [8]. From the perusal of experimental results, it is evident that the values of blackgram growth components *viz.,* plant height, Leaf Area Index and DMP at varied stages of crop growth were significantly higher with foliar application of chelated micronutrient mixture @ 0.5 per cent on 25 and 45 DAS (S_6). Foliar application of chelated micronutrient mixturehas provided availability of iron, boron for blackgram which in turn might have resulted in vigorous root, cell wall, plasma membrane, enhancement of cell division, tissue differentiation and metabolism of nucleic acid, carbohydrate and shoot initiation reflecting upon enhanced crop growth and establishment in terms of plant height. A similar inference was documented [9]. Hence, the optimum availability of these nutrients with foliar

application of chelated micronutrient mixture @ 0.5 per cent on 25 and 45 DAS (S_6) have promoted higher uptake and utilization of essential plant nutrients for enhanced photosynthates production and further crop canopy establishment. This may be the reason for resultant enhanced values of leaf area index with this treatment. Iron (Fe) enters many plant enzymes that play dominant roles in oxidoredox reaction of photosynthesis and respiration [10]. Magnesium (Mg) has major physiological and molecular roles in plants, it is a component of the chlorophyll molecule, a co-factor for many enzyme processes associated with phosphorylation and the hydrolysis of various compounds, as well as a structural stabilizer for various nucleotides [11]. The appreciable increments in respect of plant height and leaf area index noticed in this treatment have positively reflected upon enhanced crop dry matter production. The results are in line with the findings [12] and [13]. The absence of chelated micronutrients in control (S_1) might be the reason for the lower values of growth components. Similar findings were reported [14]. Sulphur has a key role in improving crop yield and produce quality which can only be performed in sulphur deficient areas by augmenting the supply of sulphur fertilizer. For optimum growth and production, plant tissue must contain sufficient concentrations of sulphur, only then; the plants can produce carbohydrate, proteins, oils and vitamins to their full potential. Sulphur is also known to promote nodulation in legumes and thereby enhancing the nitrogen fixation. Nodule numbers plant^{-1} of blackgram was significantly higher with 20kg S ha^{-1}.The increase in nodule numbers is due to positive interaction of sulphur with phosphorus which helped in better root development and root nodulation. Moreover, Sulphur stimulates the cell division and helpful in the promotion of root nodules. The present finding is in agreement with the results [15].

CONCLUSION

On the basis of the result of the field experiment, it may be inferred that in RDF + 20 kg S ha^{-1} along with foliar application of 0.5% chelated micronutrient mixture foliar spray on 25 and 45 DAS had a remarkable effect on the growth attributes of Blackgram *cv* ADT-3.

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Table 1. Sulphur fertilization and foliar nutrition on plant height (cm) at harvest stage of blackgram

Sub plot treatment	Main plot treatment			
	M_1 (RDF alone)	**M_2** (RDF+15 kg S ha^{-1})	**M_3** (RDF+20 kg S ha^{-1})	**Mean**
S_1-(Control)	22.00	24.83	24.97	23.93
S_2-(2 per cent DAP foliar spray on 25 and 45 DAS)	33.66	48.07	53.75	45.16
S_3-(0.5 per cent MAP foliar spray on 25 and 45 DAS)	27.825	39.37	39.46	35.55
S_4-(2 per cent Urea foliar spray on 25 and 45 DAS)	30.82	42.35	45.21	39.46
S_5-(2 per cent Water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS)	27.93	39.41	39.51	35.62
S_6-(0.5 per cent Chelated micronutrient mixture foliar spray on 25 and 45 DAS)	36.51	50.91	56.62	48.01
Mean	29.79	40.82	43.25	37.95
	Main	Sub	M × S	S × M
SE_d	0.34	0.75	1.23	1.30
CD (p=0.05)	0.93	1.53	2.59	2.66

Table 2. Sulphur foliar nutrition on Leaf area index at flowering stage of blackgram

Sub plot treatment	Main plot treatment			
	M_1 (RDF alone)	**M_2** (RDF+15 kg S ha^{-1})	**M_3** (RDF+20 kg S ha^{-1})	**Mean**
S_1-(Control)	1.63	1.91	1.93	1.82
S_2-(2 per cent DAP foliar spray on 25 and 45 DAS)	2.76	4.11	4.60	3.82
S_3-(0.5 per cent MAP foliar spray on 25 and 45 DAS)	2.19	3.26	3.30	2.91
S_4-(2 per cent Urea foliar spray on 25 and 45 DAS)	2.50	3.60	3.85	3.32
S_5-(2 per cent Water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS)	2.22	3.29	3.33	2.95
S_6-(0.5 per cent Chelated micronutrient mixture foliar spray on 25 and 45 DAS)	3.02	4.35	4.85	4.07
Mean	2.39	3.42	3.64	3.15
	Main	Sub	M × S	S × M
SE_d	0.02	0.04	0.07	0.07
CD (p=0.05)	0.06	0.08	0.14	0.14

Table 3. Sulphur fertilization and foliar nutrition on number of branches plant^{-1} at harvest stage of blackgram

Sub plot treatment	Main plot treatment			
	M1 (RDF alone)	**M2** (RDF+15 kg S ha^{-1})	**M3** (RDF+20 kg S ha^{-1})	**Mean**
S1-(Control)	2.11	2.41	2.45	2.32
S2-(2 per cent DAP foliar spray on 25 and 45 DAS)	3.71	5.93	6.81	5.48
S3-(0.5 per cent MAP foliar spray on 25 and 45 DAS)	2.88	4.54	4.64	4.02
S4-(2 per cent Urea foliar spray on 25 and 45 DAS)	3.31	5.11	5.51	4.64
S5-(2 per cent Water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS)	2.90	4.59	4.68	4.06
S6-(0.5 per cent Chelated micronutrient mixture foliar spray on 25 and 45 DAS)	4.12	6.38	7.23	5.91
Mean	3.17	4.83	5.22	4.40
	Main	Sub	M × S	S × M
SEd	0.03	0.57	0.96	0.99
CD (p=0.05)	0.08	0.12	0.20	0.20

Table 4. Sulphur fertilization and foliar nutrition on dry matter production(kg ha^{-1}) at harvest stage of blackgram

Sub plot treatment	Main plot treatment			
	M1 (RDF alone)	**M2** (RDF+15 kg S ha^{-1})	**M3** (RDF+20 kg S ha^{-1})	**Mean**
S1-(Control)	1033.83	1232.19	1261.81	1175.94
S2-(2 per cent DAP foliar spray on 25 and 45 DAS)	1811.58	2743.66	3098.19	2551.14
S3-(0.5 per cent MAP foliar spray on 25 and 45 DAS)	1432.16	2156.12	2201.15	1929.81
S4-(2 per cent Urea foliar spray on 25 and 45 DAS)	1641.31	2398.12	2569.39	2202.93
S5-(2 per cent Water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS)	1456.04	2187.67	2213.85	1952.52
S6-(0.5 per cent Chelated micronutrient mixture foliar spray on 25 and 45 DAS)	1985.85	2913.93	3265.46	2721.75
Mean	1560.13	2271.95	2434.97	2089.02
	Main	Sub	M × S	S × M
SEd	14.05	26.50	44.20	45.90
CD (p=0.05)	39.02	54.12	93.54	93.74

Table 5. Sulphur fertilization and foliar nutrition on root length (cm) at harvest stage of blackgram.

Sub plot treatment	Main plot treatment			
	M1 (RDF alone)	**M2** (RDF+15 kg S ha^{-1})	**M3** (RDF+20 kg S ha^{-1})	**Mean**
S1-(Control)	7.85	9.01	9.05	8.64
S2-(2 per cent DAP foliar spray on 25 and 45 DAS)	12.59	18.89	21.29	17.59
S3-(0.5 per cent MAP foliar spray on 25 and 45 DAS)	10.20	15.01	15.29	13.50
S4-(2 per cent Urea foliar spray on 25 and 45 DAS)	11.42	16.48	17.63	15.18
S5-(2 per cent Water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS)	10.24	15.13	15.31	13.56

S_6-(0.5 per cent Chelated micronutrient mixture foliar spray on 25 and 45 DAS)	13.76	20.12	22.63	18.84
Mean	11.01	15.77	16.87	14.55
	Main	Sub	M × S	S × M
SE_d	0.09	0.18	0.30	0.32
CD (p=0.05)	0.26	0.37	0.64	0.65

Table 6. Sulphur fertilization and foliar nutrition on root nodules plant^{-1}at harvest stage of blackgram.

Sub plot treatment	**Main plot treatment**			
	M_1 (RDF alone)	**M_2** (RDF+15 kg S ha^{-1})	**M_3** (RDF+20 kg S ha^{-1})	**Mean**
S_1-(Control)	12.83	14.45	14.51	14.45
S_2-(2 per cent DAP foliar spray on 25 and 45 DAS)	19.73	28.59	32.01	26.78
S_3-(0.5 per cent MAP foliar spray on 25 and 45 DAS)	16.22	23.16	23.33	20.90
S_4-(2 per cent Urea foliar spray on 25 and 45 DAS)	18.01	25.09	26.85	23.32
S_5-(2 per cent Water soluble fertilizer (19:19:19) foliar spray on 25 and 45 DAS)	16.28	23.28	23.39	20.98
S_6-(0.5 per cent Chelated micronutrient mixture foliar spray on 25 and 45 DAS)	21.45	30.29	33.70	28.48
Mean	17.68	24.14	25.63	22.48
	Main	Sub	M × S	S × M
SE_d	0.14	0.28	0.46	0.48
CD (p=0.05)	0.38	0.58	0.97	0.98

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RESEARCH ARTICLE

Electrical and Dielectric Study of Epoxy Resin

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ABSTRACT

This paper studies the electrical conductivities of an Epoxy resin. Epoxies have been present from the 1890's but their popularity soared in the 1900's. The requirement to expand it's usage across industries has driven different projects that aimed to modify these compounds with a beneficial outcome. Many papers were published that show research in the electrical conductivities of a diverse set of epoxy systems. Some of them include the study of mechanical, thermal and their electrical properties. The previous research helps us to understand the electrical properties of the above said compounds by changing the ratio of resin to hardener as well as with the addition of distinct fillers have given insight on its applications. On the other hand addition of nano-fillers such as silica, boron nitride and silicon nitride affect the dielectric properties of epoxies. The D.C. conductivity measurements were performed using two probe method. Dielectric response of the compound was investigated in the frequency range, 20 Hz to 1MHz by LCR meter. Dielectric permittivity (€'(w)) and Dielectric loss factor (€''(w)) were investigated. It was observed that (€'(w)) and (€''(w)) decrease with increase in frequency at all temperatures.

Keywords: Electrical conductivity, Epoxy Resin, Dielectric permittivity, Dielectric loss factor, LCR Meter

INTRODUCTION

Epoxy Resins which are also known as epoxide resins, polyepoxides or ethoxyline resins are the most commonly used thermosetting polymers. They are a class of prepolymers and polymers that have epoxide groups. These substances are generally characterized by the existence of 1,2-epoxy groups per molecule. The epoxide group is sometimes referred to as the oxirane group. The Epoxy resin would be a highly viscous and a pourable liquid without silica. Aerospace was the first official industry epoxies entered in the 1950's and over the next two decades they were commercialised. Mostly found in aerospace, automotive and construction industries, they exhibit high strength and durability along with flexibility. Epoxy resins have spread to other sectors due to their insulating and preservative properties. Their application now range from being used in metal coatings, paint manufacturing to electronics, high-tension electrical insulators electrical components, LEDs and adhesives for structural and other purposes. Reaction of epoxy resins with themselves or with any poly-functional hardeners forms a thermosetting polymer which is found to have favorable mechanical properties with high thermal and chemical resistance. They have high dielectric strength, excellent adhesion to various substrates, chemical and solvent resistance, effective electrical insulation along with a wide range of operating temperatures. They are resistant to many chemicals like sulfuric acid, acetone, methanol, sodium hydroxide, and organic acids depending on the formulation. They tend to wet surfaces easily, making them especially suitable for composite applications [1]. Generally epoxies can be bisphenol-based, novolaks, aliphatic, halogentaed, diluents or Glycidylamine. Diglycidyl ether bisphenol A (DGEBA) which is made from phenol and acetone and diglycidyl ether bisphenol F (DGEBF) made of phenol and formaldehyde are the two most common epoxy resins used today. They are sold as liquids, solid resins, resins dissolved in solvent, etc. Epoxy resin is also used to modify several polymers such as polyurethane or unsaturated polyesters to enhance their physical and chemical attributes.

Curing

The curing process is the major key to understand the electrical properties of Epoxy resins [2]. Uncured epoxy resins tend to exhibit poor chemical, mechanical and heat resistant properties. On curing they undergo an exothermic process and hence temperatures are increased step by step as to control rate of curing and avoid excessive heat build-up [3]. Epoxy resins can be cross-linked with themselves through the process of catalytic homo-polymerisation or with co-reactants such as phenols, alcohols, thiols (mercaptans), poly-functional amines, anhydrides and acids. Co-reactants are known as hardeners or curatives. The process of mixing epoxy compounds with curatives in specific ratios to achieve the final result is called curing. On curing an an epoxy resin it transforms from a low molecular weight compound to a three-dimensional chemical structure. Some epoxy resin-hardener combinations cure at ambient temperatures, a lot more require heat with temperatures around 150°C to 200°C (392°F). Insufficient heat during the process of curing results in a compound with incomplete polymerization which creates a substance with reduced mechanical, chemical and heat resistance. Cure temperature should attain the glass transition temperature (T_g) of the fully cured network as to achieve maximum properties. Hardeners are referred to as latent hardeners when they react with epoxy resins at elevated temperatures despite having minimal or limited reactivity at room temperature. It is useful for many industrial operations to use latent hardeners because they allow the epoxy resin and hardener to be combined and stored for some time before use. The epoxy curing reaction can be accelerated by adding small amounts of accelerators. Tertiary amines, carboxylic acids and alcohols (especially phenols) are efficient accelerators. Bisphenol A is a highly effective and widely used accelerator, but is now increasingly replaced due to health concerns with this substance. Electrical conductive adhesives are available in one-part and two-part systems. The electronics industry refers to the one-part system as''liquid–powder system'' and two-part system as the ''liquid–liquid system''. The one-part system is made up of rather intricate mixes of surfactants, colloidal fillers, surfactants, and epoxy resin [4].

Electrical Properties

For epoxy-based composites electrical properties can be examined via direct current (dc) conductivity and broadband dielectric spectroscopy (BDS) measurements. The applicability range of BDS (depends on devices) is 10^{-6}

to 10^{12} Hz.The conductivity of polymers has been increased over the last decades but the majority of the polymers remain insulating in nature. Epoxy resins also belong to this class of having low conductivity due to limited amount of free charge carriers. This is the reason their applications are found as protective coatings in electrical circuit components in microelectronics, as insulating adhesives in multilayered capacitors, etc. As it is an insulator, it is a dielectric so application of electric field produces permanent and induced dipoles that are forced to be aligned in the direction of field. Polarization creates a net positive and net negative surface charge at the edges of the dielectric material. The dielectric permittivity of epoxy resins are increased quickly at low frequencies and high temperatures. The alternation of the applied field is low at low frequencies which give time to dipoles to be aligned with the field. Dielectric permittivity and polarization are proportional quantities. So polarisation is expected to be maximum at lower-frequencies and higher-temperatures [5]. It is known that epoxy resins undergo a transition from a glassy to rubbery at a specific temperature called as glass transition temperature (T_g). Different values of it can exist for the same polymer corresponding to completion, midpoint and onset of transition. As temperatures approach T_g the macromolecular chains which were rigid start to move due to the thermal energy offered. This facilitates the alignment of dipoles in epoxy resins parallel to electric field, increasing polarisation and the real part of dielectric permittivity. For determining ac conductivity the variation of epoxy's conductivity vs frequency and temperature are seen. It assumes lower values cause of resin's insulating nature. Also, there is a strong dispersion with frequency and temperature exhibited in the conductivity spectra. At lower frequencies, influence of temperature seems to be more. In isothermal conditions, σ_{ac} has constant values approaching its dc value, but above a specific frequency, ac conductivity shows an exponential dependency on frequency. This is mostly common in disordered systems and is in accordance with the ac universality law [6]. Capacitance is defined as the ability of a substance or device to storage energy. These values can be attained through the usage of an LCR meter. In a cured epoxy system, the dielectric constant changes with temperature, frequency and filler. Fillers contribute to a major role in epoxy resin formulations. The characteristics to take in consideration on addition of fillers are volume fraction of filler, particle characteristics (size, shape, surface area…), filler ratio, strength of filler, adhesion of filler to the resin, viscosity of the base resin and toughness of the base resin [7]. For example, a system can have a dielectric constant that increases with temperature for a 75-Hz application, but fluctuates with temperature for a 1000Hz application. Usually, the dielectric constant increases with higher temperatures and decreases with higher frequencies. Essentially, epoxies show better insulation properties for higher frequencies but lose some of their insulation capabilities at higher temperatures. Addition of mineral filler can increase the dielectric constant of a particular epoxy system slightly. The Dissipation Factor is generally found to be 0.003 to 0.03 at 1 KHz and up to 0.05 at 1MHz. At ambient temperatures, it generally increases as the frequency gets higher. As temperature rises, the effect on dissipation factor varies greatly depending on the operating frequency and the specific chemistry. To limit the heating of the material and the effect on the nearby circuit, a smaller dissipation factor is preferred [8].

MATERIALS AND METHODS

The materials were acquired and transformed into pellets with the usage of appropriate techniques in the lab. Resin and hardener failed to hold a rigid structure when made into pellets individually. Therefore the M-seal compound consisting of resin and hardener were mixed in the same ratio and devised into a pellet of thickness 3.77mm and radius 0.7625cm (diameter 1.525cm). The pellet was coated with silver paint for the detection of electrical properties and this sample was left to air dry for 24 hours to achieve results with maximum accuracy. The sample was determined with Wayne Kerr 41100 digital LCR meter interfaced to a computer as shown in the figure. Data was acquired as a function of temperature with variation of frequencies. The Capacitance attained from the measurements was converted to dielectric constant using the relation

$\varepsilon = C/C_O$ ———(1)

where 'C' is the measured capacitance in Farads. C_O is evaluated from the relation

$C_o = \varepsilon_o * (A/t)$ ———(2)

Where 'ε_O' is the permittivity of free space ($8.854*10^{-12}$ F/m), 'A' is the area of cross-section of the sample and 't' is the thickness of the sample in S.I units. Cured epoxy systems show variation in dielectric constants on basis of temperature, frequency and filler concentration. When dielectric substances are subjected to ac voltage, there is dissipation in the form of heat due to absorption of electrical energy by the material [9].

RESULTS AND DISCUSSION

Electrical Investigation

One of the most crucial properties of a conducting polymer is electrical investigation, especially when looking into their potential application in electrical devices. An attempt was made to measure D.C Conductivity for different temperature. The powder was compressed into pellets for electrical characteristics using uniaxial pressing.

D.C. Conductivity

Using a two-probe conductivity technique, room-temperature conductivity is assessed on a pressed pellet. DC Conductivity was measured by the formula

$\sigma_{dc} = (L / R* A)$ ---------- (3)

Where 'R' is the resistance, 'L' is the thickness of the pellet and 'A' is the area of cross section of the pellet. It's value is found to be 25.94MΩ.

Dielectric measurement:

For dielectric measurement the capacitance (C) and the dissipation factor (D) for the sample was measured using an Wayne kerr 41100 LCR meter in the frequency range of 20Hz – 500KHz. Figures 1 & 2 represents the variation of dielectric constant (**ε'(**w**)**) and dissipation factor of the sample composite (for different Temperatures). At low frequencies the sample exhibited high dielectric constant which decreased with increase in frequency. It is observed that the dielectric constant is independent of frequency beyond 100 KHz. In dielectric analysis dielectric constant at high frequencies is associated with dipolar relaxation and at low frequencies; the dielectric constant is associated with interfacial polarization and d.c. conductivity. At high frequencies the variation in the field is very rapid for dipoles to align themselves hence less dielectric constant. For the present sample composite the dielectric constant (35°C) is 3.76E-29 at 20 KHz and it is around 3.25E-29 at 100 KHz. Figures 3 & 4 show variation of dielectric constant and Dissipation factor as a function of temperature at various frequencies. It is observed that both ε'(w) and dissipation factor increases with increase in temperature. This is because as the temperature of the sample increases the dipoles comparatively become free and they respond to the applied electric field. Hence polarization increases which leads to increase in dielectric constant [10,11].

CONCLUSION

The results for Dielectric constant and Dielectric loss of Epoxy Resin were determined at the frequency range of 20Hz-500 KHz with temperature varied from 35°C to 115°C using an LCR meter (Wayne Kerr 41100). The sample composite exhibited increased values of both dielectric constant and dissipation factor with decrease in frequency and increase in the temperature. It has been studied that variation in temperature on composite showed a prominent effect on conductivity and dielectric properties.

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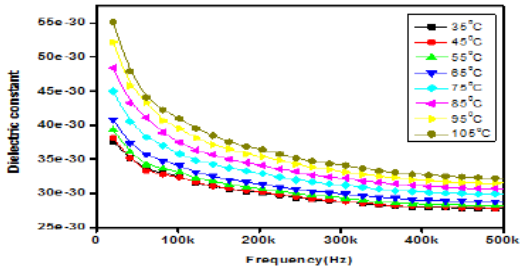

Figure 1. Dielectric constant of the sample as a function of frequency

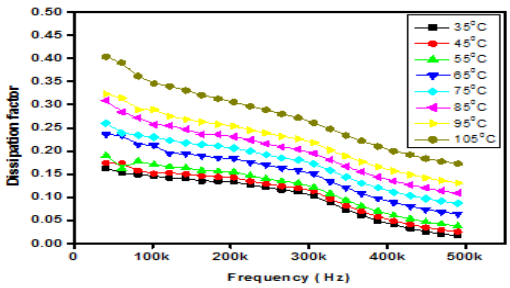

Figure 2. Dissipation factor of the sample as a function of frequency

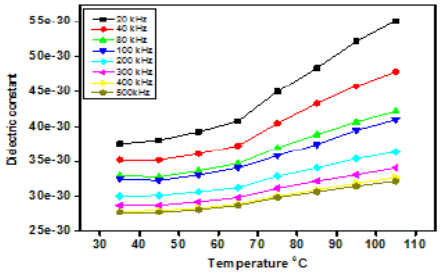

Figure 3. Dielectric constant of the sample as a function of Temperature

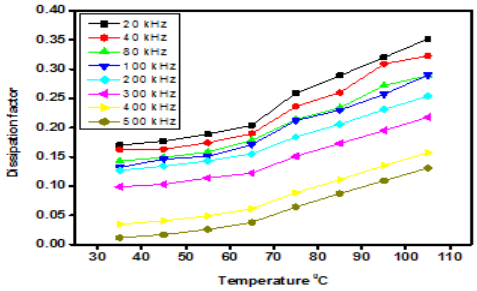

Figure 4. Dissipation factor of the sample as a function of Temperature

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RESEARCH ARTICLE

Inventory Model with Eco-Centric Cost Parameters in Processing Industries

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ABSTRACT

New industries are evolving in recent times and one among them is the processing industries that stand distinct from the modalities of the existing discrete processing. The resultant products of these processing industries are the assortment of several raw materials that demand different magnitudes of processing. Uninterrupted production systems devoid of dearth, product quality and environmental sustainability are the major concerns of every industry and processing industries are not an exception to it. With the aim of fulfilling the requirements of these industries, a deterministic inventory model is formulated with different types of cost parameters pertaining to quality maintenance and environmental promotion. The developed model also encompasses the costs associated with bio-friendly processing technology. The optimal order quantity is determined using an analytical method. The efficacy of the newly framed model is substantiated with secondary data and it is observed that the incorporation of specific costs pertaining to quality and eco-sustainability makes this model reflect the social responsibility of these industries.

Keywords: Processing industries, environment, inventory model, optimization, quality.

INTRODUCTION

The manufacturing industries around the globe are ebbing out with new strategies to proliferate their annual profit in manifolds. The predominant manufacturing modalities are discrete and process and they are delineated in different dimensions of production. Discrete manufacturing comprises a sequence of processes involving assembling of parts to form a final product. The resultant product under discrete manufacturing is reversible in nature and the industries that produce automobiles, electronic products, furniture and so on are a few illustrations of discrete manufacturing industries. Process manufacturing is yet another manufacturing process consisting of product production from various input materials in different proportions based on formulae. The resultant products of process manufacturing are irreversible. Chemical processing, food processing are some of the familiar instances of processing industries. Both discrete and process manufacturing industries battle challenges in production and in mitigating environmental impacts caused by adopting production technology devoid of the component of eco-friendliness. Optimization of production expenses is one of the primary objective of any processing industries, but at recent times the environment has been festered by the industrial growth and as an immediate consequence of it, the environmental protection activities are made coercive to make these industries reflect their social and environmental concern in product production system, distribution and disposal mechanism. The inclusion of environmental costing increases the financial burden of these industries. One of the best measures to leaven such financial constraints of the production sectors is to formulate inventory models with the objectives of cost minimization and profit maximization which will certainly smoothen the production system and promote environmental sustainability simultaneously. Economic Order Quantity model by Harris [1] and Economic Production Quantity model by Taft [2] are the two fundamental inventory models developed with the objective of finding the optimal order quantity. These inventory models are later extended to other optimizing models with the inclusion of different concepts of shortages with backorders [3], deterioration [4], inflation [5,6] ,trade credit [7]. In addition to these models, rework models, reverse logistics models, supply chain models are also developed to find optimal solutions to the production problems of manufacturing sectors. As the environmental concern of the industries are equally increasing along with their economic concerns, Maurice Bonney [8] has formulated an inventory model encompassing the social cost parameters and this model caused a paradigm shift in inventory modelling.

Based on the Enviro- inventory model development, many inventory models are developed by the researchers with the inclusion of environmental sustainable cost parameters in various aspects such as pollution control costs, carbon emission costs, green costs, green supply chain costs, transportation costs, disposal costs, product recovery cost, rework cost, recycling cost. The inclusion of these costs make the inventory model both economic and environmental oriented. The literature on inventory models with the association of the aspects of sustainability, green supply chain and environmental orientation comprises several inventory models out of which the models related to this research work are presented as follows. Arslan et al [9], Marchi et al [10], Sarkar et al [11], Tiwari et al [12] proposed sustainable inventory models with the inclusion of sustainable cost parameters. Biswajit Sarkar [13] has discussed environmental impact in sustainable inventory management. Hovelaque et al [14], Sangal et al [15], Ma´rquez et al [16] have developed an inventory model with the inclusion of carbon emission cost parameters. Rani et al [17] have framed green supply chain inventory models. Bazan [18] developed environmental centric remanufacturing inventory model with. Nivetha et al incorporated the attributes of industrial ecology in inventory model. Toles [19] proposed quantity discount inventory model with environmental cost parameters. Ritha et al [21-25] developed more environmentally concerned inventory model with the annexation of different costs associated with environmental sustainability and conservation. In these environmental oriented inventory models the environmental cost parameters are pertinent to the aspects of either the terminal activities or the mediating activities but not stage wise production activities. The environmental costing must comprise of the costs associated with all the activities involved in product production. In addition to quality sustenance of the product the quality maintenance of the production system is equally important for conserving the environment. The sustainability cost parameters discussed in the above models focus on the external cost of environmental sustainability. Green supply chain costs comprise of green transportation costs, pollution abatement costs, carbon emission mitigating costs. These costs are

certainly the external cost that constraints the production sectors, but they are not the only costs of environmental sustainability. The internal costs of conserving the environment has to be taken into consideration as the quality of the production system is the deciding factor of the quality of the product produced and the quantity of waste generation. The inspection and supervision right from the beginning to the end of product production involves quality sustenance costs and the inclusion of such cost parameters will certainly add to the internal component of environmental sustainability. It is found that these inventory models lack the internal component of environmental cost parameters. The objective of environmental sustainability shall be achieved only with the inclusion of both internal and external cost components of the environment. To begin with, the internal environmental cost components of the production system have to be discussed and this is one of the research gaps found in the above models considered. Also in the above inventory models the inclusion of environmental costs pertains to discrete production systems and to the best of our awareness the existence of inventory models with eco-friendly strategies and their respective cost parameters with special focus on process industries are very limited. As the process industries are highly flourishing at recent times and henceforth inventory models pertinent to these kinds of process industries in specific have to be developed and this research work bridges the gaps with the formulation of a process inventory model with the inclusion of internal cost components of environment sustainability. The paper is segmented into four components. Section 2 comprises model development, section 3 presents the numerical example to corroborate the proposed model and section 4 concludes the work.

MODEL DEVELOPMENT

Let us consider the product production system of a processing industry with production rate F and demand rate J. Let Q(t) symbolizes the inventory level during the time period $[0,\lambda]$ and the differential equations that represents the level of inventory at any time t belonging to $[0,\lambda]$ is presented as below along with deterioration rate of c in the time interval $[\lambda_1, \lambda]$.

$\frac{dQ(t)}{dt} = F - J \quad$ for $0 \le t \le \lambda_1$ (2.1)

$= -J \quad$ for $\lambda_1 \le t < \lambda$ (2.2)

With the boundary conditions as $Q(0) = Q(T) = 0$. (2.3)

The value of Q(t) in $0 \le t \le \lambda_1$ is (F-J)t and in $\lambda_1 \le t < \lambda$ is $\frac{J}{c}\left[e^{c(T-t)} - 1\right]$ is obtained using the above differential equations.

The value of I_{max} is $J\left[1 - \frac{J}{F}\right]T$ (2.4)

The production system requires different raw materials as input say M_n where $n \in N$. But naturally not the whole of the raw materials enter the production processes, a certain portion say β_n of M_n do not enter the production process and gets turned out to be waste. The total purchasing cost of the raw materials is $\sum_n M_n Cm_n$ where Cm_n is the unit purchasing cost of the each of the nraw materials. The product production involves a sequence of m processing steps which requires m machines at each stage of processing. The maintenance cost of the machines for each of the m machines is Ma_m respectively where $m \in N$ and the total maintenance cost is $\sum_m Ma_m$. The bio-friendly production system subjects the raw materials to pre-processing stage to enrich the quality of the resultant product. The total pre-processing cost of the raw materials is $\sum_n (1-\beta_n) M_n P_n$. Where P_n represents the pre-processing cost per unit of each n raw material. The total waste generated from the raw materials is $\sum_n \beta_n M_n$. The total treatment cost of the waste is $G\sum_n \beta_n M_n$, where G is the treatment cost per unit. The total disposal cost of the waste is $D\sum_n \beta_n M_n$, where D is the unit disposal cost. The holding cost per unit per unit of time h_1 and a special holding cost h_2 is spent per unit per unit of time in $[\lambda_1, \lambda]$ to prevent the deterioration. The total cost is the sum of initialization cost, holding cost, purchasing cost, machinery maintenance cost, pre-processing cost, waste treatment cost, waste disposal cost. The total average cost is

$$\frac{1}{T}\left[I + \sum_n Cm_n M_n + \sum_m Ma_m + \sum_n (1-\beta_n) M_n P_n + G\sum_n \beta_n M_n + D\sum_n \beta_n M_n + h_2 + \frac{h_1}{2}\left[1 - \frac{J}{F}\right] JT^2\right]$$

The goal of this model is to optimize the total average cost and hence the EPQ model is formulated as below

Min TAC (T) =

$$\frac{1}{T}\left[I + \sum_n Cm_n M_n + \sum_m Ma_m + \sum_n (1-\beta_n) M_n P_n + G\sum_n \beta_n M_n + D\sum_n \beta_n M_n + h_2\right] + \frac{h_1}{2}\left[1-\frac{J}{F}\right]JT \quad 2.5$$

Such that T > 0

The value of T that optimizes 2.5 is T* which is equal to

$$\sqrt{\frac{2\left[I + h_2 + \sum_n Cm_n M_n + \sum_m Ma_m + \sum_n (1-\beta_n) M_n P_n + G\sum_n \beta_n M_n + D\sum_n \beta_n M_n\right]}{h_1 J\left(1-\frac{J}{F}\right)}} \quad 2.6$$

And the TAC(T*) is

$$\sqrt{J\left(1-\frac{J}{F}\right)2\left[I * h_2 * \sum_n Cm_n M_n * \sum_m Ma_m * \sum_n (1-\beta_n) M_n P_n * G\sum_n \beta_n M_n * D\sum_n \beta_n M_n * h_1\right]} \quad 2.7$$

MODEL SUBSTANTIATION

The proposed model is validated with the following secondary data. Consider an inventory system with input parameters and let us Consider a production inventory system of a processing industry that produces a product with a minimum of three input raw materials. The proposed model in section 2 is validated with the following secondary data as presented in table 1. The values of M1, M2 and M3 represent the quantity of the raw materials. With the above values of the parameter, the optimal value of T* 4.69is obtained using 2.6 and the value of TAC(T*) shall be obtained using 2.7.

CONCLUSION

The process inventory model proposed in this paper is a step towards building an eco-centric model with internal cost components of environmental sustainability. The inclusion of different types of environmental costs in the inventory model associated with the stages of pre-processing and production is an added feature of this model. The developed inventory model yields optimal total average cost with many input parameters related to economic and environmental aspects. The model shall be further extended to a more comprehensive model with the inclusion of both external and internal cost components of environmental sustainability. Also the cost and demand parameters shall be discussed under fuzzy and its extended environmental forms.

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Table 1: Parameter and its values

I	h_1	h_2	M_1	M_2	M_3	Cm_1	Cm_2	Cm_3	$\beta 1$	$\beta 2$	$\beta 3$	Ma_1	Ma_2	Ma_3	P_1
300	2	3	1000 units	1500 units	1500 units	25	35	45	0.2	0.2	0.2	1200	1200	1200	6
P_2	P_3	G	D	F	J										
5	4	10	5	50000	40000										

RESEARCH ARTICLE

Introduction to Fuzzy Hypersoft Fuzzy Cognitive Maps

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ABSTRACT

Fuzzy Hyper soft sets are more comprehensive in comparison with fuzzy soft sets in making feasible decisions. This paper proposes a new genre of decision-making models by blending fuzzy hypersoft representations with fuzzy cognitive maps and this model is an extension of the fuzzy soft fuzzy cognitive map approach. The newly developed model facilitates ranking of the factors associated with any decision-making problem with special reference to the multi-dimensional approach of the factors. The model is applied to the decision making on the institutional initiatives towards the holistic development of the student community with special reference to five core and their sub-core facets. The ranking results of the initiatives based on the newly proposed model and the conventional fuzzy cognitive model are compared and the former model appears to be more feasible, compatible and accommodative. The decision-making model constructed in this research work will certainly set new openings in the field of decision-making.

Keywords: Hyper soft set, Fuzzy Soft set, Fuzzy Cognitive Maps, Decision-making, Dimensions of Holistic Growth.

INTRODUCTION

Fuzzy Cognitive Maps (FCM) developed by Kosko [1] are directed graphs with nodes and edges representing the factors and their interrelationship between them. A simple FCM is similar to Cognitive Maps introduced by Axelerod [2] is a graphical representations with edge weights as -1,0 and 1 where the values represent negative, null and positive impacts on the factors, respectively. In weighted FCM, the edge weights belong to the range [-1,1] and

this type of FCMs is widely applied to decision making. FCMs are applied to diverse decision-making situations ranging from the dimensions of society to science and technology. To mention a few, Makrinos et al [3] to make precise decisions in agriculture, Elpiniki et al [4] in map-based modelling, Nabinsapkota et al [5] in artificial intelligence, Gonzalo et al [6] in simulation and knowledge discovery, murungweni et al [7] in the analysis, of livelihood vulnerability, Marcio et al [8] in Industrial processes, Xiaodi et al [9] in electric power system. In the above-mentioned applications, the associational impacts between the factors of the decision-making problem are graphically represented and the respective connection matrix is constructed for computational purposes. There are around 9 models of FCM grouped under four domains, namely Dynamical cognitive network, Dynamic random FCM, Fuzzy cognitive network, Fuzzy time cognitive map under, domain I labelled as Time delayed and/or uncertainty circumscribed dynamic systems. Fuzzy Grey CM and Intuitionistic FCM under domain II are labelled as complete uncertain dynamic system, Rule-based FCM, Fuzzy rules incorporated with FCM under domain III labelled as Dynamical system with human interference and Evolutionary FCM under domain IV labelled as Real-time controlled dynamical system. These different types of FCM models are developed using various learning algorithms categorized under Hebbian-based methods, population based methods and hybrid learning methods. Elpiniki et al [10] have described the merits and limitations of various learning algorithms of FCM. In addition to the above models, Vasantha and Smarandache [11] have developed neutrosophic cognitive map models. Nivetha and Smarandache [12] introduced Plithogenic cognitive map models. In the above models, the representation of edge weights are of plithogenic type, which are very comprehensive in nature and use either fuzzy or intuitionistic or neutrosophic, and the types of the models are decided by such representations.

In a FCM decision-making system, the inter associations between the factors are generally represented using fuzzy values. Priya and Nivetha [13] have made an attempt at representing the inter-associational impacts using soft sets for making focussed decision making. Molodtsov [14] introduced the theory of soft sets, which are described as parameterized families. Soft sets are extensively applied to decision-making situations characterized by parameters or attributes. Arafa et al [15], Maji and Roy [16] have applied soft sets in making optimal decisions using rough mathematics. Maji [17] has extended soft sets to fuzzy soft sets and it has a wide range of applications in decision making. Cagman et al [18] has applied fuzzy soft sets in making decisions using an fs-aggregate operator. Zhicai et al [19] constructed fuzzy soft sets using ideal notions. Kong et al [20] developed fuzzy soft sets integrated with grey theory. Osman et al [21] extended fuzzy soft sets to multifuzzy soft sets. Roy et al, Dusmanta et al [22] and Muhammad et al [23] have applied fuzzy soft sets and its extended sets to make feasible decisions. Smarandache [24] generalized the theory of soft sets to hypersoft sets, where each of the parameters or the attributes has the inclusion of sub-attributes. Hypersoft sets with weightage operators was developed by Somen et al [25].Atiqe et al [26] has developed an algorithmic approach in making decisions using hypersoftsets. Ajay et al [27] constructed a COVID decision-making model with hypersoftsets. Yolcu and Ozturk [28] extended hypersoft sets to fuzzy hypersoft sets and these sets are widely applied in making optimal decisions. AdemYolcu [29] has introduced different operators with fuzzy hypersoft sets to obtain aggregate score values of the alternatives for decision making. It is observed that fuzzy hypersoft sets are more comprehensive in comparison with fuzzy soft sets and this has motivated the authors to use fuzzy hypersoft representations in fuzzy cognitive maps. The integrated focussed FCM decision making model with soft sets is extended to the FCM model with hypersoft sets representations. The decision-making on institutional initiatives towards holistic development of the student community under the five dimensions of physical, emotional, social, spiritual and mental is determined using an integrated FCM model with fuzzy soft sets. The same FCM model is extended with fuzzy hypersoft set representations in which the dimensional characterizations in the institutional perspective of each dimension is considered for making optimal decisions about the initiative. The choice of fuzzy hypersoft sets in the FCM decision-making model is the novel feature of this research work. The paper is structured as follows. section2 presents the methodology; section 3 consists of the applications of integrated fuzzy hypersoft FCM model; section 4 compares the results with conventional FCM and integrated fuzzy soft set FCM models and the last section concludes the work.

Methodology of Integrated Fuzzy Hypersoft FCM Approach

Generally, a FCM is a directed graph which is constructed to find the inter associational impacts between the factors considered for making decisions. In addition to that, FCM is also used to rank the factors of the problem considered so as to find the prime causative or influential factor. Let us consider a decision –making problem on the factors related to the development of a sustainable society. Let the factors be F1, F2, F3 and F4. The associational impacts between the factors is generally represented as a directed graph based on the expert's opinion with edge weights ranging from [-1,1]. Here, in this case, the digraph is constructed based on assumptions, for instance. The above FCM digraph is represented as a connection matrix with simple edge weights. To determine the impact of the factor F1 on the other factors, the factor F1 is kept in ON position and the respective instantaneous vector of the form S1 = (1 0 0 0) is considered. An instantaneous vector will generally be of the form (a1,a2,..an) whereas takes the values either 1 or 0 representing ON and OFF positions respectively. The vector S1 is passed onto the connection matrix and the resultant vector thus obtained is S2 = (0 1 0 1). The vector S2 is a threshold by assigning the value 1 if the values are greater than equal to one and 0 otherwise. Also, the assumed ON position of the factor takes the value 1.The updated vector takes the form (1 10 1). By passing the vector onto the connection matrix, the vector obtained is S3 = (2 1 11) the threshold the vector becomes (1 1 11), by repeating in the same fashion and after the threshold, the final vector obtained is (1 1 11) and this is the fixed point of the dynamical FCM system. It is very evident that the factor F1 has an associational impact between all the other factors. The other factors shall be subjected to the same procedure to determine the associational impacts.

The same steps should be applied to the connection matrix with weighted edge weights. In the above procedure of conventional FCM, the associational impacts between one factor and another are determined in general sense not in specifics. To find specific associational impacts between the factors, fuzzy soft set representations shall be made. In that case, the dimensions under which the factors influence one over anotheris determined. The generalized integrated soft set FCM representation is given as follows. In this case, the factors are A1,A2,..An and the dimensions are E1,E2,..Em. The edge weights representing the associational impact of one factor on another will be expressed in terms of the dimensions considered. Let us consider the previous case of the problem of sustainable society. If the associational impact between four factors is to be discussed under three dimensions, namely E1,E2 and E3 then the connection matrix will be represented as follows The soft set representations shall be made to construct FCM and this more accommodative in making focussed decision making. The above integrated fuzzy soft set FCM representation should be extended to fuzzy Hypersoft set FCM representations by considering sub dimensions under each of the dimensions. Each of the dimensions shall be sub should categorized into sub-dimensions. For instance,E1 = { E^11, E^21,E^31}, E2 = { E^12, E^22,E^32}, E3 = { E^13, E^23}. The dominant sub-dimension under each of the dimensions shall be considered. Let us consider the dominant sub-dimensions to be {E^11, E^22, E^23}, then the connection matrix becomes This type of representation of FCM with fuzzy Hypersoft sets makes the decision-making process more specific. In this FCM the dominant sub-dimensions are considered, but it may not be the case at all times. It is necessary to consider the possible combinations of all the sub-dimensions so as to make more comprehensive decisions and this is the added feature of fuzzy Hypersoft set representations.

Applications of integrated fuzzy hypersoft set FCM model

In this section, the example considered for explaining the integrated fuzzy soft set FCM model is extended to illustrate integrated fuzzy hypersoft set FCM. In the former integrated FCM approach, the decision-making problem is to rank the initiatives taken by the higher educational institutions towards the holistic development subjecting to the dimensions of Physical Emotional, Social, Spiritual and Mental. This problem is discussed with the inclusion of sub-dimensions subjected to each of the dimensions as stated in Table 3.1. These sub-dimensions are considered as sub-attributes of the core five attributes. The possible combinations of the sub-dimensions are presented as follows in Table 3.2. The associational impacts betweenA1 and each of the factors from A1 to A10 with reference to the above 12 combinations of sub-dimensions as given in Table 3.2 is presented in Table 3.3 and the same shall be obtained for other factors. The fuzzy hypersoft representations of the connection matrix between the factors concerning to the sub-dimensions are as follows. The associational impacts between the factors shall be determined by subjecting to the

possible combinations of sub-attributes and the factors are also ranked in accordance to it. By applying the conventional procedure the factors are ranked accordingly and it is tabulated in Table 3.4

Comparative Analysis

In this section the ranking results obtained using integrated fuzzy hypersoft FCM approach is compared with integrated fuzzy soft set FCM approaches and conventional FCM free from dimensional approaches. The ranking frequencies of the factors are presented in Table 4.1. The final ranking on the core factors is made based on the frequency of rankings using CF, Integrated fuzzy soft set FCM and Integrated fuzzy hypersoft set FCM . The first five ranks of the factors are presented in Table4.2 and these factors are considered as most significant factors. It is also observed that the same ranking results are obtained by considering the ranking results obtained only considering Integrated fuzzy hypersoft set FCM. Table 4.3 presents the same. The above ranking results are obtained only with the intervention of the results of Integrated fuzzy hypersoft set FCM . It is very evident that the hypersoft set representations are more feasible in making optimal decisions.

CONCLUSION

This paper introduces the integrated Fuzzy Hypersoft set Fuzzy Cognitive Maps. The newly developed decision-making model is applied to a decision-making situation of finding the core initiatives to be taken by the higher educational institutions towards-holistic development. The proposed model is compared with models of conventional fuzzy cognitive maps and the integrated Fuzzy soft set Fuzzy Cognitive Maps. The results obtained using the proposed model are compared with other decision models and it is found that the ranking results are more convincing in the proposed model of Fuzzy Hypersoft set Fuzzy Cognitive Maps. The research work shall be extended using other extended forms of FCM such as intuitionistic FCM, neutrosophic and plithogenic FCM.

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Table 1. To determine the impact of the factor F1 on the other factors

	F1	F2	F3	F4
F1	0	1	0	1
F2	1	0	1	0
F3	0	0	0	1
F4	1	0	0	0

Table 2. The generalized integrated soft set FCM representation is given as follows.

	A1					A2					.....					.....					An				
	E1	E 2	E 3	E …	E m	E 1	E 2	E 3	E …	E m	E 1	E 2	E 3	E …	Em	E 1	E 2	E 3	E …	E m	E 1	E 2	E 3	E …	E m
A1																									
A2																									
. .																									
An																									

Table 3. namely E1,E2 and E3 then the connection matrix will be represented as follows

	F1			F2			F3			F4		
	E1	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**	**E1**	**E2**	**E3**
F1	0	0	0	…	…	…	….	…	…	…	…	…
F2	…	…	…	0	0	0	…	…	…	…	…	…
F3	…	…	…	….	…	…	0	0	0	…	…	…
F4	…	…	…	….	…	…	…	….	…	0	0	0

Table 4. Let us consider the dominant sub-dimensions to be {$E^{1}1$, $E^{2}2$, $E^{2}3$}, then the connection matrix becomes

	F1			F2			F3			F4		
	$E^{1}1$	**$E^{2}2$**	**$E^{2}3$**	**$E^{1}1$**	**$E^{2}2$**	**$E^{2}3$**	**$E^{1}1$**	**$E^{2}2$**	**$E^{2}3$**	**$E^{1}1$**	**$E^{2}2$**	**$E^{2}3$**
F1	0	0	0	…	…	…	….	…	…	…	…	…
F2	…	…	…	0	0	0	…	…	…	…	…	…
F3	…	…	…	….	…	…	0	0	0	…	…	…
F4	…	…	…	….	…	…	…	….	…	0	0	0

Table 5. Sub-Dimensions of Decision-making

Dimensions of Holistic Growth of Students	**Dimensional Characterizations in institutional perspective**
Physical (D1)	**Psychomotor activities** (P1), self-care (P2)
Emotional (D2)	**life coping skills** (E1), emotional agility (E2), mindfulness lessons (E3)
Social (D3)	Assertiveness skills (So1), **interpersonal relationship**(So2)
Spiritual (D4)	Introspection (Sp1), **values** (Sp2), empathy (Sp3)
Mental (D5)	**Positivity**(M1), self-esteem (M2), integrity (M3)

Table 6. Combinations of Sub-dimensions

η_1	(P1, E1, S01,Sp2, M1)
η_2	(P1, E1, S02,Sp2, M1)
η_3	(P1, E3, S01,Sp2, M1)
η_4	(P1, E3, S02,Sp2, M1)
η_5	(P2, E1, S02, Sp1, M3)
η_6	(P2, E1, S02, Sp2, M3)
η_7	(P2, E2, S02, Sp1, M3)
η_8	(P2, E2, S02, Sp2, M3)

η_9	(P1, E2, S01, Sp3, M2)
η_{10}	(P1, E2, S01, Sp3, M3)
η_{11}	(P1, E3, S01, Sp3, M2)
η_{12}	(P1, E3, S01, Sp3, M2)

Table 7. Associational Impact between the factors and A1 with respect to different combinations

	A1											
	η_1	η_2	η_3	η_4	η_5	η_6	η_7	η_8	η_9	η_{10}	η_{11}	η_{12}
A1	0	0	0	0	0	0	0	0	0	0	0	0
A2	.4	.2	.6	.3	.5	.4	.7	.5	.6	.7	.5	.3
A3	.3	.5	.6	.2	.7	.2	.3	.4	.6	.3	.8	.1
A4	.3	.4	.2	.5	.5	.6	.7	.8	.3	.4	.1	.5
A5	.6	.4	.3	.8	.7	.6	.4	.5	.6	.1	.3	.4
A6	.4	.5	.7	.1	.4	.2	.5	.6	.2	.6	.3	.6
A7	.6	.3	.8	.5	.3	.5	.2	.1	.5	.7	.5	.5
A8	.5	.6	.1	.6	.7	.4	.1	.3	.8	.6	.3	.6
A9	.1	.7	.4	.2	.5	.6	.8	.5	.7	.5	.2	.2
A10	.3	.5	.6	.8	.1	.3	.5	.4	.1	.3	.7	.5

Table 8. The fuzzy hypersoft representations of the connection matrix between the factors concerning to the sub-dimensions are as follows

A1($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(0,0,0,0,0,0,0,0,0,0,0,0,),A2/(.4,.2,.6,.3,.5,.4,.7,.5,.6,.7,.5,.3),A3/(.3,.5,.6,.2,.7,.2,.3,.4,.6,.3,.8,.1),A4/(.3,.4,.2,.5,.5,.6,.7,.8,.3,.4,.1,.5),A5/(.6,.4,.3,.8,.7,.6,.4,.5,.6,.1,.3,.4),A6/(.4,.5,.7,.1,.4,.2,.5,.6,.2,.6,.3,.6),A7/(.6,.3,.8,.5,.3,.5,.2,.1,.5,.7,.5,.5),A8/(.5,.6,.1,.6,.7,.4,.1,.3,.8,.6,.3,.6),A9/(.1,.7,.4,.2,.5,.6,.8,.5,.7,.5,.2,.2),A10/(.3,.5,.6,.8,.1,.3,.5,.4,.1,.3,.7,.5)}
A2($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.5,.6,.1,.5,.3,.4,.2,.1,.3,.5,.4,.6),A2/(0,0,0,0,0,0,0,0,0,0,0,0),A3/(.3,.6,.8,.6,.2,.1,.4,.5,.6,.2,.1,.4),A4/(.4,.6,.5,.7,.2,.1,.8,.3,.5,.2,.1,.5),A5/(.7,.5,.7,.2,.3,.2,.1,.6,.4,.5,.6,.8),A6/(.4,.6,.7,.5,.2,.1,.8,.5,.6,.2,.8,.9),A7/(.5,.7,.3,.1,.8,.5,.4,.3,.5,.3,.6,.7),A8/(.7,.5,.3,.1,.2,.4,.3,.6,.8,.7,.4,.3),A9/(.2,.5,.8,.5,.4,.2,.7,.8,.3,.1,.2,.9),A10/(.6,.7,.4,.7,.2,.8,.1,.9,.3,.2,.5,.6)}
A3($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.4,.8,.6,.3,.1,.2,.6,.7,.2,.3,.5,.2),A2/(.3,.6,.1,.3,.2,.5,.4,.7,.9,.6,.4,.5),A3/(0,0,0,0,0,0,0,0,0,00,0),A4/(.2,.6,.7,.7,.5,.3,.6,.8,.3,.5,.4,.1),A5/(.7,.6,.5,.3,.2,.1,.5,.4,.8,.2,.1,.3),A6/(.6,.3,.1,.8,.4,.6.5,.2,.6,.3,.2,.8),A7/(.5,.7,.6,.8,.2,.3,.6,.7,.8,.4,.5,.4),A8/(.3,.6,.7,.6,.4,.3,.5,.7,.4,.3,.2,.6),A9/(.6,.4,.7,.2,.6,.4,.2,.8,.9,.4,.2,.7),A10/(.5,.8,.4,.2,.3,.8,.2,.1,.7,.6,.3,.4)}
A4($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.3,.7,.3,.4,.5,.2,.4,.5,.6,.7,.1,.5),A2/(.7,.8,.4,.5,.2,.7,.4,.7,.3,.2,.5,.1),A3/(.4,.6,.7,.5,.1,.3,.7,.2,.1,.5,.3,.7),A4/(0,0,0,0,0,0,0,0,0,0,0,0),A5/(.5,.6,.1,.3,.6,.4,.7,.4,.8,.4,.5,.1),A6/(.6,.1,.3,.7,.8,.2,.6,.5,.2,.3,.7,.5),A7/(.5,.3,.6,.5,.9,.7,.5,.2,.6,.3,.6,.1),A8/(.1,.5,.3,.6,.5,.7,.3,.8,.4,.2,.4,.5),A9/(.4,.5,.6,.7,.9,.3,.2,.1,.5,.4,.2,.5),A10/(.3,.7,.6,.7,.5,.8,.2,.6,.3,.1,.5,.4)}
A5($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.3,.6,.4,.7,.2,.5,.6,.3,.2,.1,.4,.3),A2/(.7,.5,.3,.2,.1,.6,.5,.2,.3,.2,.7,.4),A3/(.6,.4,.5,.2,.3,.8,.5,.4,.2,.7,.1,.3),A4/(.4,.5,.6,.8,.2,.4,.5,.2,.3,.6,.4,.7),A5/(0,0,0,0,0,0,0,0,0,0,0,0),A6/(.6,.3,.5,.6,.7,.2,.1,.7,.8,.4,.5,.4),A7/(.5,.7,.9,.3,.2,.1,.5,.3,.2,.4,.6,.1)A8/(.6,.1,.5,.7,.2,.3,.7,.8,.3,.1,.3,.4),A9/(.3,.5,.7,.4,.8,.5,.3,.2,.1,.6,.4,.5),A10/(.1,.5,.2,.8,.4,.6,.3,.5,.6,.4,.3,.2)}
A6($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/5,.7,.4,.8,.3,.5,.1,.2,.4,.5,.7,.1),A2/(.1,.4,.8,.6,.5,.4,.7,.6,.2,.1,.7,.5,.4),A3/(.5,.6,.7,.4,.2,.3,.8,.6,.4,.3,.2,.6),A4/(.3,.6,.7,.5,.1,.5,.3,.8,.4,.8,.2,.1),A5/(.1,.6,.4,.9,.6,.4,.2,.1,.6,.5,.3,.2),A6/(0,0,0,0,0,0,0,0,0,0,0,0),A7/(.5,.7,.3,.8,.2,.6,.1,.5,.4,.3,.2,.7),A8/(.5,.6,.4,.7,.3,.2,.9,.8,.1,.4,.7,.8),A9/(.7,.3,.5,.7,.1,.4,.5,.3,.2,.6,.4,.2),A10/(.3,.6,.8,.9,.2,.1,.7,.4,.5,.3,.8,.4)}
A7($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.5,.3,1,.7,.4,.8,.2,.4,.6,.7,.2,.5),A2/(.6,.4,.3,.8,.6,.7,.5,.4,.2,.1,.8,.3),A3/(.5,.1,.4,.3,.8,.2,.7,.6,.5,.3,.6,.2),A4/(.3,.7,.3,.5,.2,.7,.8,.4,.1,.5,.6,.4),A5/(.2,.6,.3,.2,.1,.3,.7,.5,.4,.7,.2,.3),A6/(.3,.2,.5,.7,.1,.6,.3,.5,.7,.8,.5,.7),A7/(0,0,0,0,0,0,0,0,0,0,0,0),A8/(.5,.7,.6,.8,.3,.2,.4,.7,.4,.8,.3,.5),A9/(.6,.8,.3,.5,.6,.2,.7,.1,.4,.3,.7,.3),A10/(.7,.2,.5,.8,.2,.4,.6,.3,.7,.3,.4,.1)}

A8($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.6,.7,.3,.7,.2,.5,.4,.8,.1,.5,.6,.3),A2/(.5,.4,.2,.5,.8,.7,.3,.2,.6,.7,.5,.4),A3/(.7,.5,.6,.3,.2,.5,.1,.8,.4,.3,.6,.7),A4/(.3,.1,.7,.4,.5,.8,.7,.4,.3,.5,.6,.5),A5/(.4,.6,.3,.7,.1,.5,.6,.3,.7,.1,.5,.3),A6/(.2,.7,.4,.6,.3,.8,.4,.5,.7,.9,.7,.1),A7/(.7,.5,.3,.5,.1,.4,.2,.8,.6,.4,.1,.5),A8/(0,0,0,0,0,0,0,0,0,0,0,0),A9/(.6,.7,.8,.4,.3,.7,.2,.1,.6,.7,.5,.8),A10/(.3,.5,.6,.2,.7,.8,.5,.2,.5,.4,.3,.5)}
A9($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.7,.5,.3,.8,.5,.4,.6,.1,.3,.2,.8,.5),A2/(.3,.2,.5,.1,.6,.7,.8,.3,.5,.7,.4,.1),A3/(.5,.7,.2,.8,.3,.5,.4,.7,.3,.9,.5,.3),A4/(.8,.3,.6,.5,.7,.3,.2,.6,.4,.1,.5,.4),A5/(.7,.5,.3,.6,.1,.6,.8,.2,.4,.3,.7,.2),A6/(.3,.2,.7,.5,.4,.3,.7,.5,.2,.1,.7,.6),A7/(.6,.7,.1,.8,.2,.3,.6,.2,.1,.5,.4,.5),A8/(.1,.3,.7,.5,.2,.5,.4,.8,.9,.1,.5,.3),A9/(0,0,0,0,0,0,0,0,0,0,0,0),A10/(.2,.7,.6,.5,.3,.8,.5,.2,.1,.6,.7,.5)}
A10($\eta_1,\eta_2,\eta_3,\eta_4,\eta_5,\eta_6,\eta_7,\eta_8,\eta_9,\eta_{10},\eta_{11},\eta_{12}$)	{A1/(.5,.7,.9,.3,.2,.4,.7,.1,.5,.3,.7,.1),A2/(.7,.3,.6,.2,.1,.5,.6,.4,.7,.8,.9,.8),A3/(.3,.5,.2,.7,.8,.4,.3,.2,.6,.5,.3,.7),A4/(.1,.4,.7,.3,.6,.8,.2,.4,.5,.3,.7,.4),A5/(.6,.8,.5,.2,.1,.5,.7,.5,.3,.8,.9,.3),A6/(.5,.7,.6,.3,.2,.9,.8,.4,.6,.3,.2,.5),A7/(.3,.6,.8,.9,.6,.7,.3,.2,.1,.7,.6,.3),A8/(.8,.5,.7,.1,.4,.3,.8,.9,.4,.3,.2,.7),A9/(.5,.6,.8,.5,.3,.2,.1,.6,.7,.4,.5,.4),A10/(0,0,0,0,0,0,0,0,0,0,0,0)}

Table 9. Ranking of the factors based on fuzzy Hypersoft set representations

Factors	Ranking of the factors with respect to sub-dimensions											
	η_1	η_2	η_3	η_4	η_5	η_6	η_7	η_8	η_9	η_{10}	η_{11}	η_{12}
A1	8	6	5	9	2	6	5	10	6	4	7	5
A2	2	2	2	9	9	8	10	4	6	7	3	2
A3	5	3	9	3	3	5	3	1	1	5	8	7
A4	10	4	8	5	1	2	6	4	5	8	5	3
A5	3	8	5	4	3	4	7	7	10	9	4	9
A6	7	1	3	1	10	10	8	2	8	3	7	4
A7	4	10	10	6	8	7	4	8	3	1	8	10
A8	9	6	5	7	5	1	9	2	4	2	6	1
A9	6	5	7	2	7	3	1	8	9	10	2	6
A10	1	8	1	8	6	9	2	6	2	5	1	8

Table 10: Ranking frequencies of the factors

Factors		Rank (R) and its Frequency (F) with Reference (RE) to Conventional FCM(CF), Integrated fuzzy soft set FCM (E1,E2,E3,E4,E5), Integrated fuzzy hypersoft set FCM (η_1, η_2, η_3,η_4,η_5,η_6, η_7, η_8,η_9,η_{10},η_{11},η_{12})									
A1	**R**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**
	F	-	2	1	2	3	4	2	1	1	2
	RE		E3, η_5	E2	E1,η_{10}	η_3,η_7,η_{12}	E5,η_2,η_6,η_9	CF,η_{11}	η_1	η_4	E4,η_{10}
A2	**F**	_	4	3	3	_	1	1	2	2	2
	RE		$\eta_1,\eta_2,\eta_3,\eta_{12}$	E5, CFη_{12},	E3,E4, η_8		η_9	η_{10}	E2,η_6	η_4,η_5	E1,η_7,
A3	**F**	2	_	5	_	4	1	4	1	1	_
	RE	η_8,η_9		E5,$\eta_2,\eta_4\eta_5,\eta_7$		E1,η_1,η_6,η_{10}	CF	E2,E3,E4, η_{12}	η_{11}	η_3	
A4	**F**	3	2	2	2	4	1	_	3	_	1
	RE	E2,E4,η_5	CF,η_6	E3,η_{12}	η_2,η_8	E5,η_4,η_9,η_{11}	η_7		E1,η_3,η_{10}		η_1

A5	**F**	_	_	2	3	2	1	3	2	3	2
	RE			η_1,η_5	η_4,η_6,η_{11}	CF,η_3	E3	E1,η_7,η_8	E5,η_2	E4,η_{10},η_{12}	E2,η_9
A6	**F**	3	1	3	1	1	_	2	3	2	2
	RE	E5,η_2,η_4	η_8	E4,η_3,η_{10}	η_{12}	E2		$\eta_1,\eta_{11},$	E3,η_7,η_9	E1,CF	η_5,η_6
A7	**F**	2	2	1	2	_	2	1	4	1	3
	RE	CF, η_{10}	E1, E5	η_9	η_1,η_7		E2,η_4	η_6	E4,η_5,η_8,η_{11}	E3	η_2,η_3,η_{12}
A8	**F**	3	2	1	1	2	4	1	1	2	
	RE	E3,η_6,η_{12}	η_8,η_{10}	E2	η_9	η_3,η_5	E1,E4,η2,η11	η_4	CF	η_1,η_7	E5
A9	**F**	1	3	2	1	2	2	3	1	2	1
	RE	η_7	E4,η_4,η_{11}	E1, η_6	CF	E3,η_2	η_1,η_{12}	E5,η_3,η_5	η_8	E2,η_9	η_{10}
A10	**F**	4	3	_	_	2		_	3	2	2
	RE	E1,η_1,η_3,η_{11}	E2,η_7,η_9			E4,η_{10}	η_5,η_8		η_2,η_4,η_{12}	E5,η_6	E3, CF

Table 11. Overall Ranking of the Core factors

I	II	III	IV	V
A10	**A2**	**A3**	**A5**	**A4**

Table 12. Ranking of the Core factors using Integrated fuzzy hypersoft set FCM

I	II	III	IV	V
A10	**A2**	**A3**	**A5**	**A4**

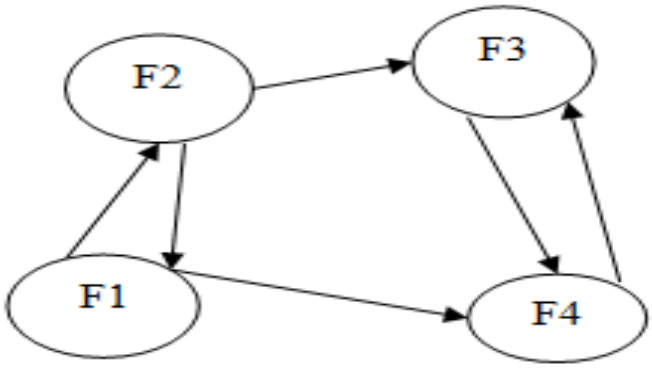

Fig. 1. Let the factors be F1, F2, F3 and F4.

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RESEARCH ARTICLE

A New Extension of Quasi Sujatha Distribution with Properties and Applications using Time Dependent Covariates

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ABSTRACT

In this paper, we have introduced a new generalization of Quasi Sujatha distribution known as Area biased Quasi Sujatha distribution. The newly introduced distribution has two parameters. The different statistical properties, Reliability measures, order statistics, moments, Bonferroni and Lorenz curves are derived. The method of maximum likelihood estimator is also used for estimating the parameters of the newly introduced distribution and also the Fisher's Information matrix have been discussed. Finally, an application of the new distribution is established by analysing a real life data set for examining its usefulness.

Keywords: Weighted distribution, Quasi Sujatha distribution, Maximum likelihood estimation, Reliability measures, Order statistics and Time dependent covariates.

INTRODUCTION

The study of weighted distributions introduced by Fisher (1934) provides a suggestive approach by using the several techniques to the unknown weight function when the classical distributions were not giving the appropriate results. The idea of weighted distributions was later formalized in a unified concept by Rao (1965) to identify the various sampling situations that can be modelled by weighted distributions. The weighted distributions arise when the observations generated from a stochastic process are not given equal chances of being recorded; rather they are recorded according to some weight function. When the weight function depends only on the length of units of interest, the resulting distribution is called as length biased distribution. Length biased concept was firstly given by Cox (1969) and Zelen (1974). The concept of weighted distributions helps us to deal with model specification and data interpretation problems. In the study of distribution theory, weighted distributions are useful because it

provides a new approach of existing standard probability distributions and also it provides methods for extending existing standard probability distributions for modelling lifetime data due to introduction of additional parameter in the model which creates flexibility in their nature. Weighted distributions occur in modeling clustered sampling, heterogeneity, and extraneous variation in the data set. The weighted distributions are mainly applied in many fields such as reliability, biomedicine, ecology, analysis of family data, meta-analysis and analysis of intervention data for the improvement of proper statistical models. Weighted distributions are milestone for efficient modelling of statistical data and prediction when the standard distributions are not appropriate. Mudasir and Ahmad (2015) studied the structural properties of length biased Nakagami distribution. Subramanian and Rather (2020) studied the length biased shanker distribution with application of Real Life data. Rather and Subramanian (2018) discussed the characterization and estimation of length biased weighted generalized uniform distribution. Quasi Sujatha distribution is a newly introduced life-time model proposed by Shanker (2016) and the proposed quasi Sujatha distribution is a particular case of Sujatha distribution. Its different mathematical and statistical properties including its moments, skewness, kurtosis, coefficient of variation, index of dispersion, hazard rate function, mean residual life function, stochastic ordering, Bonferroni and Lorenz curves and stress strength reliability have been derived and studied. Shanker (2016) have also studied the Sujatha distribution and its applications and studied its various characteristics.

Time-Dependent Covariate

A covariate in a Cox proportional hazards model is said to be a static measurement or one that changes over period. The time dependent covariate model should be employed when the predictor changes value of the observed time frame. A covariate in a Cox proportional hazards model is an each static measure or one that changes over time. The time dependent covariate model is employed the predictor changes of the value of across the noticeable time frame. Analyse the impact of smoking on survival of an example of a time-dependent covariate. Assume we have a group of people who would be contacted at annual intervals. For example data on current smoking status (defined as smoking any cigarettes in the previous month) and the estimated total number of cigarettes smoked over the previous year are obtained as portion of the interview procedure. The hypothesis that will be examined. The present cigarette smoking raises the chance of dying. Using a step-function which is equals to one if the individual is smoking at the last follow-up and zero if the individual was not smoking in last follow-up is the most direct solution. For example, an individual alive after 4 years of follow-up whom smoked at baseline, at years 1 and 2, but not at years 3 and 4, would have a time-dependent covariate equal to 1 until the starting of year 3 and subsequently goes to zero. Take note of the stage where the smoking status was altered. A step function is one that accepts constants at regular intervals.

Area Biased Quasi Sujatha (ABQS) Distribution

The probability density function of quasi Sujatha distribution (QSD) with parameters θ and α is given by

$$f(x;\theta,\alpha)=\frac{\theta^2}{\alpha\theta+\theta+2}\left(\alpha+\theta x+\theta x^2\right)e^{-\theta x};\ x>0,\theta>0,\alpha>0 \qquad (1)$$

and the cumulative distribution function of quasi Sujatha distribution is given by

$$F(x;\theta,\alpha)=1-\left(1+\frac{\theta x(\theta x+\theta+2)}{\alpha\theta+\theta+2}\right)e^{-\theta x};x>0,\theta>0,\alpha>0 \qquad (2)$$

A non-negative random variable X is said to have a weighted distribution, if the pdf of weighted random variable X_w is given by

$$f_W(x)=\frac{w(x)\,f(x)}{E(w(x))},\ x>0.$$

Where $w(x)$ be a non-negative weight function and $E(w(x))=\int w(x)\,f(x)dx<\infty.$

For different weighted models, we have different choices of the weight function $w(x)$. When $w(x) = x^c$, the resulting distribution is termed as weighted distribution. In this paper, we have to obtain the Area biased version of quasi Sujatha distribution, so we will take $c = 2$ in weights x^c, in order to get the Area biased quasi Sujatha distribution and the pdf of Area biased quasi Sujatha distribution is given by

$$f_a(x) = \frac{x^2 f(x)}{E(x^2)} \tag{3}$$

Where $E(x^2) = \int_0^\infty x^2 f(x)dx$

$$E(x^2) = \frac{2\alpha\theta + 6\theta + 24}{\theta^2(\alpha\theta + \theta + 2)} \tag{4}$$

By substituting equations (1) and (4) in equation (3), we will obtain the probability density function of Area biased quasi Sujathadistribution as

$$f_a(x) = \frac{x^2\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x} \tag{5}$$

and the cumulative distribution function of Area biased quasi Sujatha distribution is obtained as

$$F_a(x) = \int_0^x f_a(x)dx$$

$$F_a(x) = \int_0^x \frac{x^2\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}dx$$

$$F_a(x) = \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\int_0^x x^2\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}dx$$

$$F_a(x) = \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha\int_0^x x^2 e^{-\theta x}dx + \theta\int_0^x x^3 e^{-\theta x}dx + \theta\int_0^x x^4 e^{-\theta x}dx\right)$$

Put $\theta x = t \Rightarrow \theta dx = dt \Rightarrow dx = \frac{dt}{\theta}$, As $x \to x, t \to \theta x,\ x \to 0, t \to 0$

After simplification of the above equation, we will obtain the cumulative distribution function of Area biased quasi Sujatha distribution which is given by

$$F_a(x) = \frac{1}{2\alpha\theta + 6\theta + 24}\left(\alpha\theta\gamma(3, \theta x) + \theta\gamma(4, \theta x) + \gamma(5, \theta x)\right) \tag{6}$$

Reliability measures

In this section, we will obtain the survival function, hazard rate and Reverse hazard rate function of Area biased quasi Sujatha distribution.

Survival function

The survival function is also known as reliability function and the survival function of Area biased quasi Sujatha distribution is obtained as

$$S(x) = 1 - F_a(x)$$

$$S(x) = 1 - \frac{1}{2\alpha\theta + 6\theta + 24}\left(\alpha\theta\gamma(3, \theta x) + \theta\gamma(4, \theta x) + \gamma(5, \theta x)\right)$$

Hazard function

The hazard function is also known as hazard rate or instantaneous failure rate or force of mortality and the hazard function of Area biased quasi Sujatha distribution is given by

$$h(x) = \frac{f_a(x)}{S(x)}$$

$$h(x) = \frac{x^2\theta^4\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}}{\left(2\alpha\theta + 6\theta + 24\right) - \left(\alpha\theta\gamma(3, \theta x) + \theta\gamma(4, \theta x) + \gamma(5, \theta x)\right)}$$

Reverse hazard function

The reverse hazard function of Area biased quasi Sujatha distribution is given by

$$h_r(x) = \frac{f_a(x)}{F_a(x)}$$

$$h_r(x) = \frac{x^2\theta^4\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}}{\left(\alpha\theta\gamma(3, \theta x) + \theta\gamma(4, \theta x) + \gamma(5, \theta x)\right)}$$

Statistical Properties

In this section, we will discuss some statistical properties of Area biased quasi Sujatha distribution especially moments, harmonic mean, MGF and characteristic function.

Moments

Let X denotes the random variable of Area biased quasi Sujatha distribution with parameters θ and α, then the r^{th} order moment $E(X^r)$ about origin of Area biased quasi Sujatha distribution is obtained as

$$E(X^r) = \mu_r' = \int_0^\infty x^r f_a(x)dx$$

$$E(X^r) = \int_0^\infty x^r \frac{x^2\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}dx$$

$$= \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\int_0^\infty x^{r+2}\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}dx$$

$$= \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha\int_0^\infty x^{r+2}e^{-\theta x}dx + \theta\int_0^\infty x^{r+3}e^{-\theta x}dx + \theta\int_0^\infty x^{r+4}e^{-\theta x}dx\right)$$

$$= \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha\int_0^\infty x^{(r+3)-1}e^{-\theta x}dx + \theta\int_0^\infty x^{(r+4)-1}e^{-\theta x}dx + \theta\int_0^\infty x^{(r+5)-1}e^{-\theta x}dx\right)$$

After simplification of the above equation, we will obtain

$$E(X^r) = \mu_r' = \frac{\theta\left(\alpha\Gamma(r+3) + \Gamma(r+4) + \Gamma(r+5)\right)}{\theta^r\left(2\alpha\theta + 6\theta + 24\right)} \quad (7)$$

Substitute*r* = 1,2,3 and 4 in equation (7), we will get the first four moments of Area biased quasi Sujatha distribution.

$$E(X) = \mu_1' = \frac{(6\alpha + 24 + 120)}{\left(2\alpha\theta + 6\theta + 24\right)}$$

$$E(X^2) = \mu_2' = \frac{(24\alpha + 120 + 720)}{\theta\left(2\alpha\theta + 6\theta + 24\right)}$$

$$E(X^3) = \mu_3' = \frac{(120\alpha + 720 + 5040)}{\theta^2\left(2\alpha\theta + 6\theta + 24\right)}$$

$$E(X^4) = \mu_4' = \frac{(720\alpha + 5040 + 40320)}{\theta^3\left(2\alpha\theta + 6\theta + 24\right)}$$

$$\text{Variance} = \frac{(24\alpha + 120 + 720)}{\theta\left(2\alpha\theta + 6\theta + 24\right)} - \left(\frac{(6\alpha + 24 + 120)}{(2\alpha\theta + 6\theta + 24)}\right)^2$$

$$S.D(\sigma) = \sqrt{\frac{(24\alpha + 120 + 720)}{\theta(2\alpha\theta + 6\theta + 24)} - \left(\frac{(6\alpha + 24 + 120)}{(2\alpha\theta + 6\theta + 24)}\right)^2}$$

$$C.V = \frac{\sigma}{\mu_1'} = \sqrt{\frac{(24\alpha + 120 + 720)}{\theta(2\alpha\theta + 6\theta + 24)} - \left(\frac{(6\alpha + 24 + 120)}{(2\alpha\theta + 6\theta + 24)}\right)^2} \times \frac{(2\alpha\theta + 6\theta + 24)}{(6\alpha + 24 + 120)}$$

Harmonic mean

The harmonic mean for the proposed Area biased quasi Sujatha distribution can be obtained as

$$H.M = E\left(\frac{1}{x}\right) = \int_0^\infty \frac{1}{x} f_a(x)dx$$

$$= \int_0^\infty \frac{x\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}dx$$

$$= \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\int_0^\infty x\left(\alpha + \theta x + \theta x^2\right)e^{-\theta x}dx$$

$$= \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha\int_0^\infty xe^{-\theta x}dx + \theta\int_0^\infty x^2e^{-\theta x}dx + \theta\int_0^\infty x^3e^{-\theta x}dx\right)$$

$$= \frac{\theta^4}{2\alpha\theta + 6\theta + 24}\left(\alpha\int_0^\infty x^{2-1}e^{-\theta x}dx + \theta\int_0^\infty x^{3-1}e^{-\theta x}dx + \theta\int_0^\infty x^{4-1}e^{-\theta x}dx\right)$$

After simplification, we obtain harmonic mean of the distribution,

$$H.M = \frac{\theta(\alpha\theta + 2\theta + 6)}{2\alpha\theta + 6\theta + 24}$$

Moment Generating Function

Moment generating function is the expected function of a random variable. As its name implies moment generating function can be used to compute the moments of a distribution. The familiar definition of moment generating function given by

$$M_X(t) = E\left(e^{tx}\right) = \int_0^\infty e^{tx} f_a(x)dx$$

Using Taylor's series, we obtain

$$= \int_0^\infty \left(1 + tx + \frac{(tx)^2}{2!} + \ldots.\right) f_a(x)dx$$

$$= \int_0^\infty \sum_{j=0}^\infty \frac{t^j}{j!} x^j f_a(x)dx$$

$$= \sum_{j=0}^\infty \frac{t^j}{j!} \mu_j'$$

$$= \sum_{j=0}^\infty \frac{t^j}{j!} \left(\frac{\theta(\alpha\Gamma(j+3) + \Gamma(j+4) + \Gamma(j+5)}{\theta^j (2\alpha\theta + 6\theta + 24)} \right)$$

$$M_X(t) = \frac{1}{(2\alpha\theta + 6\theta + 24)} \sum_{j=0}^\infty \frac{t^j}{j!\theta^j} \left(\theta(\alpha\Gamma(j+3) + \Gamma(j+4) + \Gamma(j+5))\right)$$

Similarly, the characteristic function of Area biased quasi Sujatha distribution can be obtained as

$$\varphi_X(t) = M_X(it)$$

$$M_X(it) = \frac{1}{(2\alpha\theta + 6\theta + 24)} \sum_{j=0}^\infty \frac{it^j}{j!\theta^j} \left(\theta(\alpha\Gamma(j+3) + \Gamma(j+4) + \Gamma(j+5))\right)$$

Order Statistics

Order statistics deals with the arrangement of samples in an ascending order and have been largely used in reliability and life testing. Consider $X_{(1)}$, $X_{(2)}$,…, $X_{(n)}$denote the order statistics of a random sample X_1, X_2,…,X_n from a continuous population with probability density function$f_x(x)$and cumulative distribution function $F_X(x)$, then the probability density function of r^{th} order statistics $X_{(r)}$is given by

$$f_{x(r)}(x) = \frac{n!}{(r-1)!(n-r)!} f_X(x)[F_X(x)]^{r-1}[1 - F_X(x)]^{n-r} \qquad (8)$$

Substitute equations (5) and (6) in equation (8), we will get the probability density function of r^{th}order statistics of Area biased quasi Sujatha distribution

$$f_{x(r)}(x)=\frac{n!}{(r-1)!(n-r)!}\left(\frac{x^2\theta^4}{2\alpha\theta+6\theta+24}\left(\alpha+\theta x+\theta x^2\right)e^{-\theta x}\right)$$

$$\times\left(\frac{1}{2\alpha\theta+6\theta+24}\left(\alpha\theta\gamma(3,\theta x)+\theta\gamma(4,\theta x)+\gamma(5,\theta x)\right)\right)^{r-1}$$

$$\times\left(1-\frac{1}{2\alpha\theta+6\theta+24}\left(\alpha\theta\gamma(3,\theta x)+\theta\gamma(4,\theta x)+\gamma(5,\theta x)\right)\right)^{n-r}$$

Therefore, the probability density function of higher order statistic $X_{(n)}$ of Area biased quasi Sujatha distribution can be obtained as

$$f_{x(n)}(x)=\frac{nx^2\theta^4}{2\alpha\theta+6\theta+24}\left(\alpha+\theta x+\theta x^2\right)e^{-\theta x}\left(\frac{1}{2\alpha\theta+6\theta+24}\left(\alpha\theta\gamma(3,\theta x)+\theta\gamma(4,\theta x)+\gamma(5,\theta x)\right)\right)^{n-1}$$

andthe probability density function of first order statistic$X_{(1)}$ of Area biased quasi Sujatha distribution can be obtained as

$$f_{x(1)}(x)=\frac{nx^2\theta^4}{2\alpha\theta+6\theta+24}\left(\alpha+\theta x+\theta x^2\right)e^{-\theta x}\left(1-\frac{1}{2\alpha\theta+6\theta+24}\left(\alpha\theta\gamma(3,\theta x)+\theta\gamma(4,\theta x)+\gamma(5,\theta x)\right)\right)^{n-1}$$

Likelihood Ratio Test

Let the random sample $X_1, X_2, \ldots\ldots X_n$of size n drawn from the Area biased quasi Sujatha distribution. For testing, we set up the hypothesis.

$$H_o: f(x)=f(x;\theta,\alpha) \quad \text{against} \quad H_1: f(x)=f_a(x;\theta,\alpha)$$

Whether the random sample of size $n$comes from the Area biased quasi Sujatha distribution or quasi Sujathadistribution, the following test statistic procedure is used.

$$\Delta=\frac{L_1}{L_o}=\prod_{i=1}^{n}\frac{f_a(x;\theta,\alpha)}{f(x;\theta,\alpha)}$$

$$\Delta=\frac{L_1}{L_o}=\prod_{i=1}^{n}\left(\frac{x_i^2\theta^2(\alpha\theta+\theta+2)}{2\alpha\theta+6\theta+24}\right)$$

$$\Delta=\frac{L_1}{L_o}=\left(\frac{\theta^2(\alpha\theta+\theta+2)}{2\alpha\theta+6\theta+24}\right)^n\prod_{i=1}^{n}x_i^2$$

We should reject the null hypothesis, if

$$\Delta=\left(\frac{\theta^2(\alpha\theta+\theta+2)}{2\alpha\theta+6\theta+24}\right)^n\prod_{i=1}^{n}x_i^2>k$$

Equivalently, we also reject the null hypothesis, if

$$\Delta^* = \prod_{i=1}^{n} x_i^2 > k\left(\frac{2\alpha\theta+6\theta+24}{\theta^2(\alpha\theta+\theta+2)}\right)^n$$

$$\Delta^* = \prod_{i=1}^{n} x_i^2 > k^*, \text{Where } k^* = k\left(\frac{2\alpha\theta+6\theta+24}{\theta^2(\alpha\theta+\theta+2)}\right)^n$$

For large sample of size *n*, $2log\ \Delta$ is distributed as chi-square distribution with one degree of freedom and also chi-square distribution is used for determining the *p*-value. Also, we reject the null hypothesis, when the probability value is given by

$p(\Delta^* > \lambda^*)$, Where $\lambda^* = \prod_{i=1}^{n} x_i^2$ is less than a specified level of significan ce and $\prod_{i=1}^{n} x_i^2$ is the observed value of the statistic Δ^*.

Maximum Likelihood Estimator and Fisher's Information Matrix. In this section, we will discuss the maximum likelihood estimator for estimating the parameters of Area biased quasi Sujatha distribution and also derive its Fisher's information matrix. Let X1, X2,…,Xn be a random sample of size nfrom the Area biased quasi Sujatha distribution, then the corresponding likelihood function is given by

$$L(x) = \prod_{i=1}^{n} f_a(x)$$

$$L(x) = \prod_{i=1}^{n}\left(\frac{x_i^2\,\theta^4}{2\alpha\theta+6\theta+24}\left(\alpha+\theta x_i+\theta x_i^2\right)e^{-\theta x_i}\right)$$

$$L(x) = \frac{\theta^{4n}}{(2\alpha\theta+6\theta+24)^n}\prod_{i=1}^{n}\left(x_i^2\left(\alpha+\theta x_i+\theta x_i^2\right)e^{-\theta x_i}\right)$$

The log likelihood function is given by

$$\log L = 4n\log\theta - n\log(2\alpha\theta+6\theta+24) + 2\sum_{i=1}^{n}\log x_i + \sum_{i=1}^{n}\log\left(\alpha+\theta x_i+\theta x_i^2\right) - \theta\sum_{i=1}^{n} x_i \qquad (9)$$

The maximum likelihood estimate of θ and α can be obtained by differentiating the log likelihood equation (9) with respect to parameters θ and α and must satisfy the normal equations

$$\text{s}\ \frac{\partial \log L}{\partial\theta} = \frac{4n}{\theta} - n\left(\frac{(2\alpha+6)}{2\alpha\theta+6\theta+24}\right) + \sum_{i=1}^{n}\left(\frac{x_i+x_i^2}{\left(\alpha+\theta x_i+\theta x_i^2\right)}\right) - \sum_{i=1}^{n} x_i = 0$$

$$\frac{\partial \log L}{\partial\alpha} = -n\left(\frac{2\theta}{2\alpha\theta+6\theta+24}\right) + \sum_{i=1}^{n}\left(\frac{1}{\left(\alpha+\theta x_i+\theta x_i^2\right)}\right) = 0$$

Because of the complicated form of likelihood equations algebraically, it is very difficult to solve the above non-linear system of equations. Therefore, we use the numerical technique like Newton-Raphson method for estimating the

parameters of the proposed distribution. To obtain the confidence interval we use the asymptotic normality results. We have that if $\hat{\lambda} = (\hat{\theta},\ \hat{\alpha})$ denoted the MLE of $\lambda = (\theta,\ \alpha)$. We can state the result as follows

$$\sqrt{n}(\hat{\lambda} - \lambda) \to N_2\left(0,\ I^{-1}(\lambda)\right)$$

Where $I^{-1}(\lambda)$ is the Fisher's informatio n matrix.

$$I(\lambda) = -\frac{1}{n}\begin{pmatrix} E\left(\frac{\partial^2 \log L}{\partial \theta^2}\right) & E\left(\frac{\partial^2 \log L}{\partial \theta \partial \alpha}\right) \\ E\left(\frac{\partial^2 \log L}{\partial \alpha \partial \theta}\right) & E\left(\frac{\partial^2 \log L}{\partial \alpha^2}\right) \end{pmatrix}$$

$$E\left(\frac{\partial^2 \log L}{\partial \theta^2}\right) = -\frac{4n}{\theta^2} + n\left(\frac{(2\alpha+6)^2}{(2\alpha\theta+6\theta+24)^2}\right) - \sum_{i=1}^{n}\left(\frac{\left(x_i + x_i^{\,2}\right)^2}{\left(\alpha + \theta x_i + \theta x_i^{\,2}\right)^2}\right)$$

$$E\left(\frac{\partial^2 \log L}{\partial \alpha^2}\right) = n\left(\frac{4\theta^2}{(2\alpha\theta+6\theta+24)^2}\right) - \sum_{i=1}^{n}\left(\frac{1}{\left(\alpha + \theta x_i + \theta x_i^{\,2}\right)^2}\right)$$

$$E\left(\frac{\partial^2 \log L}{\partial \theta \partial \alpha}\right) = -n\left(\frac{2(2\alpha\theta+6\theta+24) - 2\theta(2\alpha+6)}{(2\alpha\theta+6\theta+24)^2}\right) - \sum_{i=1}^{n}\left(\frac{\left(x_i + x_i^{\,2}\right)}{\left(\alpha + \theta x_i + \theta x_i^{\,2}\right)^2}\right)$$

Since λ being unknown, we estimate $I^{-1}(\lambda)$ by $I^{-1}(\hat{\lambda})$ and this can be used to obtain asymptotic confidence intervals for θ and α.

Application

In this section, we have analyzed and evaluated a life-time data set in Area biased quasi Sujatha distribution and the model has been compared with quasi Sujatha and Sujatha distributions. A real life data set is given below as. The real life data set associated with 40 (Time dependent) censoredpatients suffering from blood cancer (leukemia) is reported from one of ministry of health hospitals in Saudi Arabia (see Abouammah et al.).The data set is given below in table 1 as The estimation of model comparison criterion values along with the estimation of unknown parameters are determined by using the R software. We compare the Area biased quasi Sujatha distribution with quasi Sujatha distribution and Sujatha distribution, we insert the criterion values AIC (Akaike information criterion), AICC (Corrected Akaike information criterion) and BIC (Bayesian information criterion).The better distribution is which corresponds to lesser values of AIC, AICC, BIC and -2logL.The formulae for determination of criterion values are given as.

$$\text{AIC} = 2k - 2\log L \qquad \text{AICC} = AIC + \frac{2k(k+1)}{n-k-1} \quad \text{and} \quad \text{BIC} = k\log n - 2\log L$$

where the number of parameters are *k*in the statistical model, *n* is the sample size and *–2logL*is the maximized value of log-likelihood function under the considered model. From results given in table 2, it has been clearly observed that the Area biased quasi Sujatha distribution have the lesser AIC, AICC, BIC and -2logL values as compared to the quasi Sujatha distribution and Sujatha distribution, which witnessed that the Area biased quasi Sujatha distribution fits better than the quasi Sujatha distribution and Sujatha distribution. Hence, we can conclude that the Area biased quasi Sujatha distribution leads to a better fit than the quasi Sujatha distribution and Sujatha distribution.

CONCLUSION

In the present article, we have introduced a new model of quasi Sujatha distribution known as Area biased quasi Sujatha distribution. The newly introduced distribution is to be generated by using the Area biased technique and taking the quasi Sujatha distribution as the base of distribution. The different statistical properties of the proposed distribution have been derived. Supremacy of the new distribution in real life is established with demonstration of a real life data set and it is found from the result of a data set that the Area biased quasi Sujatha distribution fits better than the quasi Sujatha and Sujatha distributions.

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Table 1: Data regarding blood cancer (leukaemia) patients from health hospitals in Saudi Arabia

0.315	0.496	0.616	1.145	1.208	1.263	1.414	2.025	2.036
2.162	2.211	2.37	2.532	2.693	2.805	2.91	2.912	3.192
3.263	3.348	3.348	3.427	3.499	3.534	3.767	3.751	3.858
3.986	4.049	4.244	4.323	4.381	4.392	4.397	4.647	4.753
4.929	4.973	5.074	5.381					

Table 2: Shows values of ML estimates, Corresponding Standard errors, Criterion values & Performance of fitted distribution

Distributions	MLE	S.E	- 2logL	AIC	BIC	AICC
Area Biased Quasi Sujatha	$\hat{\alpha} = 3.2564807$ $\hat{\theta} = 1.3886761$	$\hat{\alpha} = 3.4933805$ $\hat{\theta} = 0.1353724$	144.7102	148.7102	152.0879	149.034
Quasi Sujatha	$\hat{\alpha} = 0.00100000$ $\hat{\theta} = 0.85935576$	$\hat{\alpha} = 0.40320188$ $\hat{\theta} = 0.09660441$	148.6236	152.6236	156.0013	152.947
Sujatha	$\hat{\theta} = 0.77001589$	$\hat{\theta} = 0.07012463$	152.9545	154.9545	156.6433	155.059

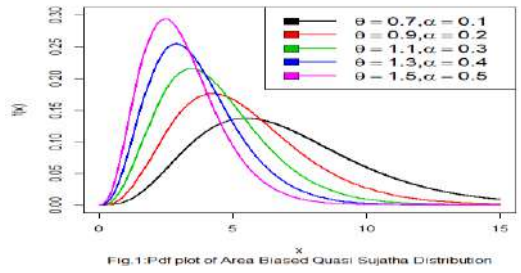

Fig.1. Pdf plot of area Biased Quasi Sujatha distribution

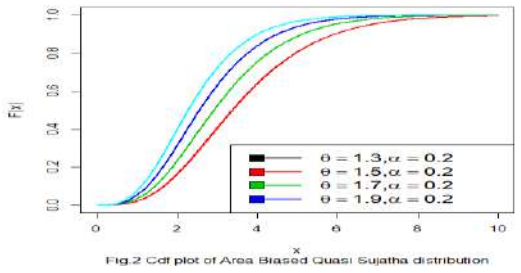

Fig. 2. Cdf plot of area Biased Quasi Sujatha distribution

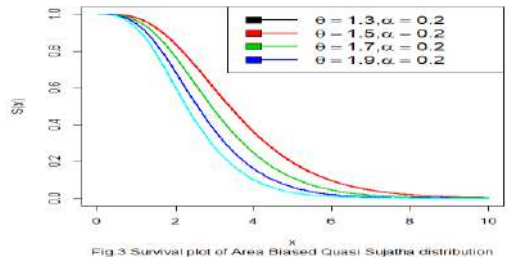

Fig. 3: Survival plot of area Biased Quasi Sujatha distribution

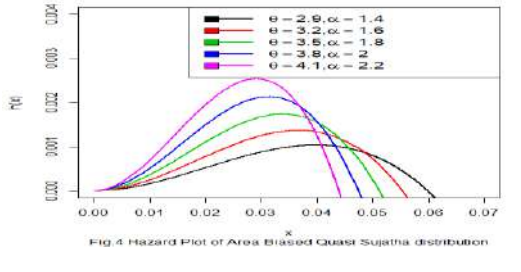

Fig. 4. Hazard plot of area Biased Quasi Sujatha distribution

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RESEARCH ARTICLE

Biomedical Sentence Classification using Fast Text Classification Techniques

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ABSTRACT

Text categorization is becoming more crucial as the amount of internet information grows. Any issue involving Text Classification (TC) requires pre-processing activities that may impact the classification accuracy. Event extraction is crucial for extracting biomedical data. Biological events are dynamic interactions between biological constituents, such as proteins and genes. The popularity of unstructured text data is increasing due to online social media. Numerous marketing applications need to organize this data at sizes unavailable to human codlings, such as identifying changes in communication sentiment or other researcher-defined content categories. Numerous approaches for categorizing unstructured Text automatically have been described. Fast Text needs minimum pre-processing, minimal hyper parameter tweaking, and is GPU-independent; optional task-specific pre-processing steps are easy and self-explanatory to create, and model training is exceptionally fast. We proposed the fastText model in categorizing biomedical phrases from the PubMed 200k and provide a pre-processing step that allows fastText to be applied to sentence sequences. Lastly, we demonstrate that our model predictions may be seen. There are experiments done on the datasets to verify the applicability of the suggested strategy. For biological TC applications, our method is ideal.

Keywords: N-Gram, Classification, FastText, Semi-Supervised Learning, Biomedical

INTRODUCTION

Fast Text is an open-source library created by Face book AI Research (FAIR) devoted only to text categorization simplification. Fast Text is a package that enables the generation of efficient word representations and provides out-of-the-box support for text categorization. In today's networked world, a great deal of text data is created worldwide. This textual data contains descriptions of objects. For instance, individuals write product evaluations on Amazon or share their comments on Face book. NLP is a computational methodology that makes use of machine learning (ML) and other methods to decipher and represent text, both verbally and in writing. NLP attempts to solve the following issues:

Modeling of a subject

In general, texts have a certain focus. Topic modelling is often used to determine the presence of "abstract topics" or hidden structures inside a collection of documents. Topic modelling is a useful tool for summarising. For example, a system like this would help the reader obtain a comprehension of the document's content and a high-level description of what's happening in a document like a legal brief. The following are the different sentence types: TC is a critical issue since it helps us to classify blobs of text. As an example, "Shahrukh Khan was on fire at a Dubai event," whereas "Fire breaks out in-store opposite Breach Candy Hospital" should be categorised as "News" by a computer system. The following sentence has been automatically translated by a computer programme: At least 3,000 languages are spoken across the globe. About a fifth of these languages are spoken by fewer than 1,000 people. Consequently, many languages are being extinguished, and with each extinguishment comes the loss of a substantial piece of our common cultural heritage. Translated by Google, it is now the finest. Though just 103 languages have been included at the time of this writing, ML translation models must be developed that can be trained with a minimum amount of inputs and display great prediction power.

Systems for asking and answering questions

Automated systems that react to natural language users' bogus questions are the aim. Closed domain systems, which can extract pertinent documents and Text, may be quite accurate.

Sentiment analysis

Sentiment analysis is the process of determining people's shared goals and intentions when they discuss something. Individuals make decisions depending on their emotions. Many people's demands are mostly emotional, and individuals are typically rather candid about feelings. Developing a system that accounts for this will always offer significant value to the firm.

Event extraction

This is useful when a large amount of data is kept in Text. For example, a "crime" may be described in legal literature as a series of "investigations" and "hearings." For instance, the "hearing" events may comprise the events of "offering arguments" and "presenting evidence".

Detection of named entities

The purpose of this system is to extract and categorize entities or particular information according to predetermined categories, such as persons, organizations, and location. Consider the following statement: "South Texas is known for its spicy food." From this information, we might infer that the "buyer" like "hot meals," as well as South Texas. If enough evidence is obtained to show that people in South Texas like spicy food, more of these meals might be supplied to them.

Relationship detection

A system for detecting relationships parses the Text and determines focus points and agents, then attempts to establish a link between them. For instance, "Mike has the flu" may be transformed into Person-[RELATION:HAS]->Disease. These relationships may then be investigated in a corporate setting to develop intelligent applications.

LITERATURE REVIEW

According to the study paper[1], an effective approach, for instance, selection, may complete half of the information discovery process. A novel instance selector based on Support Vector Machine (SVM) is proposed to eliminate noisy data. It is termed support vector-oriented instance selection. The research article [2] describes the hybridization of kNN and SVM classification methods. The one-vs-near approach has been shown to work on huge datasets, thus this is a two-stage plan. The kNN classifier is used to build a list of category neighbours, which is the first step in the learning process. The kNN uses an ordered list to determine the distance between each centroid and the second stage classifier. The dataset used to train the classifier is then narrowed down to just one category using SVM's stored neighbour list.

The research study's authors [6] design new and proactive approach to estimate Bayesian probabilities. This Bayesian classifier utilises a conditional-independence model based on an entropy-based evaluator to choose the smallest possible collection of long, non-overlapping patterns for its classifier inputs. Another unique feature of each group's probability approximation is its own. Partitioning the attribute set into many large subsets reduces the attribute set's conditional dependence on a single class, and picking frequent item sets evaluated by attribute sets that are conditionally independent reduces the attribute set's conditional reliance even more. The research study's authors [8] created a hybrid kNN and SVM classification method. SVM-NN is the name given to this combination approach. When using Bayesian vectorization, the first step is to convert training and training text documents into numerical data points. After that, the SVM's vector space is filled with data points from the training phase. A support vector for each class is then found, and any remaining training data is removed. Data points that have not yet been labelled are placed in an identical vector space to those used in the training phase to classify previously labelled data points. For each new unlabeled point in the data set, compute the average distance between each category's support vector and the new unlabeled data point. Then find the group of new unlabeled points that have the least average distance between each of their support vectors and the new data point.

The research study's authors [9] proposed a hybrid TC technique based on a Bayesian-SVM. This first dataset is separated into two sections: training and test sets. SVM accepts just numerical data as input. Thus, the Bayesian vectorization approach translates both testing and training data to numerical representations. This converts the written input to a numerical representation. The SVM classifier will then use this data as input. And the result is categorized data that may be utilized as a training set once again. The SVM classification method was presented in a research paper [11]. Classifying natural text (or hypertext) resources based on their content into specified categories is known as text classification. In a variety of scenarios, including as email filtering, internet searching, workplace automation, and news storey classification, this problem exists. Structural Risk Minimization (SRM) is a potential method for classifying data. The research study [15] demonstrated how active learning might derive rules from SVMs. This approach focuses on issue areas, which are those regions of the input space with the highest noise level for rule extraction. This approach requires a pre-processing stage in which missing values are eliminated from the data, nominal variables are encoded with weights of evidence (WOE), and the data is separated into training and test sets.

TEXT CLASSIFICATION METHODS

As implied by the name, text categorization assigns a unique class to each document in the Text. TC is shown by sentiment analysis and email categorization. Each day, millions of digital documents are created in this age of

technology. It would take an enormous amount of time and human work to classify them as spam and non-spam, significant and inconsequential, and so on**.** NLP text categorization methods come to our rescue here. Using hands-on experience with a sentiment analysis challenge, let's demonstrate how this works.

ML TEXT CLASSIFICATION TECHNIQUES

k-nearest Neighbor (kNN)

The kNN classifier uses the Euclidean distance formula most often and extensively. New unlabeled data points are compared to previously recognised data points using this method. The distance between a new input data point and its nearest neighbours is calculated as the first step in the KNN classification stage.

Naive Bayes (NB)

Based on the Bayes rule, the NB technique is a simple way to classify inputs with a high degree of complexity. Documents and classes are taken into account in this Bayes rule. Each instance of an issue, represented by a feature value vector, is given a class name by this model. An individual feature has a value that is distinct from the value of other characteristics. Prior probability and posterior probability are both known in the nave Bayesian method. To the class with the greatest possibility of receiving it, the document is provided.

SVM

Based on SRM, the Support Vector Machine (SVM) is an excellent method for classifying large datasets. It has been claimed that SVM is a more accurate classifier than prior prototypes. By maximising the classification margin, SVM determines the optimum hyperplane for classifying training data. By using a method known as the kernel approach, SVM may also be applied to points on nonlinear decision surfaces, allowing for the creation of linear separation hyperplane from the higher-dimensional feature space.

DECISION TREE

The DT shapes classification simulations by using a tree structure. The resulting tree has decision nodes and leaf nodes. DTs are capable of manipulating both category and quantitative data.

RULE-BASED CLASSIFICATION

In rule-based categorization, a set of rules are applied to data. IF-THEN rules are used to categorise items in this classification method. There are two terms used to describe this part of the rule: predicate (the right part) and antecedent (the left part). THEREFORE, the right part of the rule may be used. Each attribute test in the state's antecedent part is logically finished. In the next part, we'll discuss how to anticipate class outcomes.

BIOMEDICAL TEXT MINING TASKS

Document Retrieval: Document retrieval or information retrieval is retrieving relevant documents from a big collection. There are two fundamental retrieval methodologies used. Two types of retrieval are available: query-based and document-based. Documents that match a user-specified search term are returned. A sorted list of documents that are similar to the document of interest is returned in document-based retrieval.

The Prioritization of Documents:

In most cases, the papers that are returned are ranked in order of relevance. Biomedical journal metrics (e.g., impact factor and citation count) as well as the MeSH index for biomedical articles are used to rank content in different biological document retrieval systems. A number of measures are used to gauge the degree of similarity between two texts (e.g., Jaccard similarity, cosine similarity).Organizing and presenting data is a major part of this task. Concept extraction and relation/event extraction are the two most important parts of the information extraction process. It is possible to utilise relation/event extraction to predict the relationship or biological event between two concepts, whereas concept extraction automatically finds the ideas included in the articles. Extracting biological

information from structured data is not a simple task—Text taken from data extraction. Knowledge discovery involves the application of artificial intelligence, machine learning, pattern recognition, data mining, and statistics. Information extraction and knowledge discovery are used to build databases and pathways.

Knowledge Summarizing

Knowledge summarization aims to create information on a certain subject from a single or a collection of documents. The technique condenses the original material through content reduction, selection, and generalization to communicate the most critical essential elements.

SYSTEM MODEL

Datasets

The PubMed 200k RCT dataset was intended to serve as a baseline for categorising consecutive sentences collected from PubMed. PubMed 200k and PubMed 20k are two separate portions of the database. According to PubMed 200k, there are around 200,000 abstracts of randomised controlled trials (RCTs) that include 2,2 million sentences. The 20,000 abstracts in PubMed 20k are a shortened version of PubMed 200k. An individual dataset statement may be categorised as either "background" or "outcome" based on the kind of data it contains. There are predefined training, validation and testing data divisions. It was also decided to build a larger training set to see whether it would boost classification results. Additional structured abstracts from PubMed-indexed papers with a medical focus were added to the PubMed 200k collection. 872,000 abstracts are included in this training split of the larger corpus (compared to only 190,000 abstracts in the training split of PubMed 200k). Similar to the PubMed 200k sample, a bigger corpus was used to verify and evaluate the results. Whitespace was used to separate punctuation from words in all corpora, and lowercase letters were used to transcribe them. FastText's supervised n-gram model Biological text sentences are classified using the fastText NLP software. The fastText supervised n-gram classification model is used to classify sentences. In response to the n-grams in the embedding, each phrase embedding is learnt repeatedly. There are n-grams that represent the local word order for each sentence to be expressed in a normalised bag. With more n-gram data being fed into the network, more learnable vector embeddings may be used to enhance the fastText model, which can be thought of as a shallow neural network. GitHub was used to get the fastText library. The grid search for hyperparameters in each experiment was extensive. N-gram embedding dimensionality (10, 20, and 50 dimensions), the number of words in a word N-gram (1, 2, 3, or 4) as well as training epochs were all hyperparameters that were tested (between 1 and 8 training epochs for 20k datasets and between 1 and 4 training epochs for larger datasets). We didn't modify any of the other settings. Ubuntu 16.04 was used for all of the tests, using an Intel Core i7-6700 4x3.40 GHz CPU and 32 GB RAM running the operating system. Python 3.6 Jupyter notebooks were used to run the tests. Scikit-learn was used to conduct statistical analysis.

Hyperparameter sweeps were carried out using Jupyter notebooks, which may be seen on GitHub. Data on sentence sequences cannot be used as-is since FastText uses a simple bag of N-grams model. Our pre-processing phase for fastText takes use of the sequential pattern of sentences in PubMed abstracts by providing extra information tokens that indicate the location of a sentence within an abstract and the content of previous and succeeding phrases. This extended sentence representation is sent into fastText's bag-of-N-grams model, which is subsequently trained on the newly added tokens. N-gram lexicon and number of N-grams required to classify each sentence are both increased, yet fastText remains incredibly efficient. Each hyperparameter configuration included a pre-processing step that added numeric sentence position information, as well as representations of the performance of the two preceding and following sentences for each hyperparameter configuration. Randomly selecting terms from the PubMed 200k training corpus allowed us to create constrained training datasets that simulated scenarios when training data was scarce. The training corpus for PubMed 20k contains about the same amount of sentences as the sentences picked for training ranging from 100 to 180 000. Compared were three alternative classifier setups industry-standard fastText: a fully supervised approach of speedy texting unsupervised embeddings were learned on the whole PubMed 200k training text corpus (without labels) before moving to supervised training for sentence classification in the semi-supervised fastText model. To train Sent2Vec+ Multilayer Perceptron, the sent2vec vector representation of words and a hidden layer (size 100 neurons) from the PubMed 200k training corpus were input into MLP.

RESULTS AND DISCUSSION

When it comes to unattended and semi-supervised training, Fast Text generated remarkable results in a very short amount of time. A basic pre-processing strategy was suggested by us, however it's worth mentioning that fastText worked well without needing any further pre-processing. Hyperparameter values varied widely, yet the approach worked effectively in all of them. An important factor in the classification success was the number of time intervals (epochs) used in the classification process. Extra scripts for hyperparameter adjustment are necessary since fastText includes an in-built mechanism for early pausing. The weighted F1 scores for various models trained on single sentences is represented at table1.Using the fastText model, it is possible to describe increasingly complex structures by encoding them as new embeddable entities that may be added to the model's vocabulary. The system was able to manage the increasing number of embedded entities in context sentences because we considered each word as a different entity is represented at figure 1.

CONCLUSIONS

Only little hyperparameter adjustments are required for Quick Text, and no GPU is required for model training. Optional task specific pre-processing steps are easy to design, and the model training procedure is extremely rapid. Because of this article, we proposed fastText involving the classification of biological text. There's a lot of "rough material" that can be captured by the bag-of-N-grams approach for most classification tasks. The pre-processing algorithm's bag-of-N-grams representation may also be used to capture contextual information, such as the content of preceding and subsequent phrases and the sentence's placement within the abstract. The model described delivers high F1 scores, among other things.

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Table 1: Weighted F1 scores for various models trained on single sentences.

Model	PubMed 20k	PubMed 200k
Logistic Regression	0.838	0.862
Artificial Neural Network	0.854	0.891
Proposed Fast text model - bag-of-N-grams model	0.900	0.927

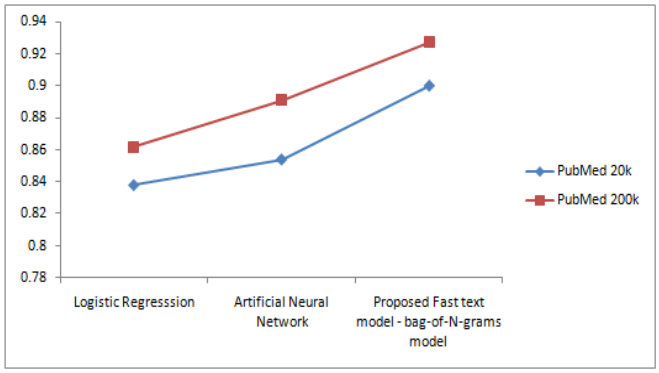

Figure 1: Fast text model –bag of – N-grams vs. existing models for F1 Score

RESEARCH ARTICLE

The Impact of Psychological Well-Being of Students Due to Online Learning

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ABSTRACT

The global epidemic situation has forced scholarly activities to move to online platforms. Students react to online learning in a variety of ways; some may see it as a positive challenge, potentially expanding learning limits and competency; others may see it as a negative pressure, potentially harming the student's psychological well-being. These days, mental health disorders are becoming more common among students. Understanding school pupils' well-being and the factors that contribute to it can aid in explaining and describing techniques that will more than likely assist children in preparing for the future. One of the most pressing problems in adolescent research is whether or not there is effective mental prosperity or psychological health among school children. The paper's goal is to investigate the impact of online learning on students' psychological well-being. This paper is exploratory in nature; data was gathered through primary sources such as questionnaires, and respondents were students in grades 9 through 12. The pandemic has brought about schools closed all around the globe and shift from traditional classroom to online platform. Based on this analysis, we discuss the paper discoveries show the psychological well-being of students on online learning. Students are exhausted with online learning. Students encountered an extent of troubles, such as emotional changes for everyday schedules, social disengagement ,new virtual learning conditions, Sleep disturbance, excessive workload, depression, anxiety, nervousness, stress among students have been commonly seen. Mental prosperity issues have

gotten progressively regular among students these days. Online learning can adversely affect the physical, mental, emotional and social well-being of school students. Students are exposed to psychological wellness issues which have resulted to major concern to the society.

Keywords: Psychological well-being, Online Learning, Mental Health, Mental prosperity.

INTRODUCTION

Academicians and students are no longer able to gather in physical classrooms due to the Corona virus outbreak. This predicament has compelled all academic activities to migrate to digital platforms, from class attendance to self-study. Learning delivered using computerized or web-based sources is known as online learning. Online learning is based on institutionalized learning and takes place via electronic devices such as computers, tablets, and even mobile phones connected to the internet. Online learning may also be defined as the sharing of ideas and information, as well as the delivery of education to a large number of students all over the world at the same time. It allows students to learn whenever, at any time, and with few restrictions. Preparing, instructing, and learning via a web-based platform on a computer, mobile phone, or other device is referred to as online learning. In this day and age, online learning and the use of internet content in K-12 is becoming more and more common. Although learning in a physical classroom is more effective since it allows for face-to-face engagement and interaction, there are numerous benefits to taking an online course, the most important of which is convenience and cost. Today, we all have internet connection, which allows us to do things like explore content for subjects to enhance our knowledge beyond what is accessible in books, and remain up to date on global current events. However, there are a few drawbacks to online learning. The most significant issue is that we receive information on a hypothetical basis, and the reality may be quite different when it comes to putting what you've learned into practise.

The effects of online learning include how technology isn't always reliable, it's more difficult for students to follow the lecture because of connectivity issues, it's more likely to be informational rather than practical, and students can't learn by doing it practically. When they are not in a physical classroom with their teachers and peers, students are easily distracted because they are having problems grasping what is being taught. Because they are subjected to more interruptions, they are unable to focus on the lecture, resulting in a lack of understanding of what is being said. Some students can have a problem with the lack of physical presence. Online learning can lead to social isolation and a lack of communication and socialisation skills among students. Psychological well-being is a combination of good feelings such as joy and working with ideal adequacy in one's self and in society. These days, mental health disorders are becoming more common among students. Students may experience difficulties with online classes as a result of the increased screen time. Students may experience more exhaustion, lack of motivation, headaches, procrastination, poor time management, and a sense of social isolation, all of which can lead to communication skills deficiencies. The pressure on students to satisfy grade requirements, retain a large amount of information, and manage their time has been shown to be the source of stress (Crocker and Luhtanen, 2003). Our urge to shift and adjust at a rapid pace has been impacted by the Corona virus, and it may be too much for us to handle.

Tension and anxiety difficulties have caused online schooling to be disrupted as a result of the epidemic. The brain and body's reaction to danger, worry, and novel situations is the source of unease. Students react to school in a variety of ways; some may regard it as a positive challenge, potentially growing their learning capacity and competency; others may see it as a negative stressor, potentially harming their mental health (Kumaraswamy, 2013).Depression is one of the most frequent psychological problems that people of all ages, especially students, face. It produces a variety of spiritual and bodily problems, and it can seriously limit a person's ability to do their job. Any traumatic event, such as missing deadlines, the death of a friend or family member, not being able to achieve what one desires, feeling worthless, or experiencing extreme stress, can lead to depression. Finally, it may cause feelings of loneliness and misery, as well as a loss of interest in the student's daily routine, and even tempt him or her to end it

all (Pompili *et al.,* 2014). Anxiety has been linked to negative behaviours among students, such as a poor eating routine, excessive pressure that causes anxiety, isolation, powerless self-perception, relational troubles, separation, and other signs of psychological wellbeing (Schofield *et al.,* 2016).

Factors of Psychological wellbeing (Carol Ryff)

Self-Acceptance

The attention to one's qualities and flaws, the optimistic point of view about self. True evaluation of one's capacities, general worth, strength and realization of fulfillment with one's self in spite of weaknesses and not thinking about past decisions.

Environmental Mastery

The capacity to select or pick alternatives from the environment utilizing physical or mental activities just as having the option to control circumstances. One creates viable utilization of opportunities and has a feeling of expertise in overseeing environmental factors and activities, including overseeing happenings and originate circumstances for the sake of advantage in individual requirements. Self-realization on one's own strength and weaknesses.

Positive Relations with Others

commitment in significant relation with others that incorporate corresponding sympathy, closeness, friendship, sensitivity and the capacity to build up steady and fulfilling deep-rooted social relations.

Personal Growth

One keeps on creating, loves new experiences, and perceives personal development in behaviour and self over the long run.

Purpose in Life

Solid objective and conviction that life holds meaning. People capabilities to set deep-rooted individual objectives and set up approaches to accomplish them.

Autonomy

Individual is free and controls their conduct autonomous of prevalent difficulties. One's capacity to keep up their skills and expertise in an assortment of social environment.

Lack of motivation, self-adequacy, and cognitive engagement, which is defined as the extent to which students are eager and able to take on the learning load, were all factors in the transition to online frameworks. This factor includes the amount of time and effort students will devote to completing the task. During the epidemic, there was a lack of attention and inspiration, which made it difficult to attend classes and study. The researchers discovered problems with web-based learning and showed worry and tension associated to the epidemic (Rajab et al.). When students were compared to the general population, the rate of tension was higher. Money-related concerns and their effects on day-to-day living, schooling disruptions, diminished social communications, and not being able to travel have all been identified as important factors impacting the student's psychological imbalance.

It was examined that the mental prosperity of students during the pandemic, were of respondents showed moderate to incredibly serious depression indications; some showed extreme tension manifestations; while some showed moderate to incredibly extreme stress and anxiety (Odriozola-González *et al.,).* It is inferred that student were for the most part stressed over the issues related with their career and examinations, and furthermore experienced frustration, uneasiness, and weariness, in an examination led among more than 30,000 students from 62 nations in isolation and the change to online learning, (Patricia A). Untreated mental issues can possibly affect social connections, efficiency and scholarly achievement (Hunt and Eisenberg, 2010). This paper tries to investigate the experience of the students in regards to psychological well-being and to find out their mental prosperity status.

Literature Review

In recent years the concept of psychological well-being has gotten extensive interest. Well- being is much of the time referred to as a societal need for government policy around the globe (Bedding ton *et al*., 2008). Nonetheless, contingent on one's expert and individual point of view, the thought of prosperity can have very many implications or connotations. It has been found common issue during every pandemic ever happened. During the 2009-2010 H1N1 flu pandemic, people of USA felt that pandemic causes weaknesses, social shame, stress, and loneliness and the people may encounter dread, tension, isolated. On a societal part, the entire local area can encounter depressed and loneliness during the pandemic. People experienced disarray, tension, and expanded dangerous practices like smoking, drinking, drug abuse, foolishness, and hazardous work rehearses due to a feeling of vulnerability. There were worried about the accessibility of basics, no works, and procedures (Alam A, 2020).

Current pandemic has impacted psychological well-being of individuals in a major way which now requires consideration from the concerned specialists to adapt to the present circumstance mentally. The discernment about this situation can likewise impact on a major part in mental prosperity (Abid Hasan Khana, Sadia Sultanaa Sahadat Hossaina). This pandemic is causing stress and anxiety due to its quick transmission of everything into online, vulnerability about the future, the financial effects, the doubt of satisfactory anticipation of the sickness and essential accessibility of heath care offices. Stress influences the unhealthy lifestyle and accordingly the danger of the covid-19 contamination grows. Peoples stress and anxiety responses brings ascend to social problematic practices, for example, individuals hurrying to stores, medical services places, medication stores, and along these lines medical care administration gets influenced (WHO, 2020).Online learning is one of the promising options in contrast to the actual schooling, still it is seen that students show a negative view on online learning.

The researcher concluded that students have begun to get exhausted with web-based learning after the initial fourteen days of learning from home, extensive tension on particular respondents whose guardians have low income, since they need to spend more to have the option to take up web-based learning, temperament or mood swings happen because of such a large number of tasks and assignments which are viewed as burden by students. The mental pressure happens during learning in online platform gives birth to stress and anxiety in students. Students started to post on social media images as grumblings against online learning, status with an assortment of objections, going from numerous assignments, quantity runs out, and the status of the internet connectivity (Hjeltnes *et al*., 2018).The online term can be an effective for everybody and it is promising a decent eventual fate of learning progress for both teacher and students if only they have been prepared a lot well with numerous abilities like quizzes, resources, online video, of educational materials to fend off students from fatigue because of a numerous assignment offered which is unquestionably a hint of student's dissatisfaction at one point it can pop up out of nowhere. Students and their families also experience these emotional wellness issues. The approach should be to carry individuals from such issues to an ordinary state, and to make them optimistic to accomplish a positive psychological wellness (Nanigopal Kapasia, Pintu Paul, Avijit Roy, Jay Saha, Ankita Zaveri, Rahul Mallick, Bikash Barman, Prabir Das, Pradip, 2020).The stress, frustration, anxiety among the American students reports that student's week economically were found to be suffering from more psychological imbalance, the shift to the online platform in view of pandemic doesn't involve peace, according to them it was really chaotic.

The vulnerability of getting the passing marks in examination was worry for some students. Schools and universities in US have sent their students home for the of the year. Closing down of campus is a mishap in academic year for the majority of the students and they feel this shut down as a worry for their academics. Students from famous Universities like Harvard might have the option to manage the monetary and scholastic down fall of a pandemic school closure, however numerous students are not ready to accept the situation. Students don't have good internet connectivity for online classes. In India as well, all the scholarly establishments have been shut down. In this current situation, the stress, frustration, anxiety, feeling of worth less among the students is rising. Due to pandemic, students and academicians are confronting pressure caused because of the unsettling influence in their personal and work lives. To adapt up in a particularly emergency situation and to keep up sound psychological well-being, both the academicians and the students are to depend on one another(Desai, 2020).Adolescence is a time of progress and

transition wherein an individual is confronted with difficulties and issues that may lead him into trouble and not allow them to make decision. Moreover, it is a period where youth get ready for life ahead. Understanding the wellbeing of teenagers and the elements that add to it will help towards explaining and characterizing approaches to all the more likely assist the youth with getting ready for future. One of the most important question that has acquired interest in the researches among youths is whether there is effective mental prosperity or general wellbeing among teenagers. (Roothman, Kirsten and Wissing, 2003).

A very few examinations have found to the positive view on psychological wellness (Herrman 2012; Wade *et al.* 2012). During our literature review we found over quite few numbers of papers on the psychological prosperity of students during an online learning. The psychological well-being has given an account of the aftereffects of horrible and posttraumatic stress problems on society. This paper is to evaluate the tension, frustration, depression, stress and anxiety of students during online learning flare-up and the explanations behind such tension, frustration, depression, stress and anxiety.

METHODOLOGY

This is a primary data-based study, the questionnaire was such designed that it was simplified to such an extent that the students can respond by using their devices. We developed a structural questionnaire of 27 statement keeping in mind the mental state and academic difficulty factors, students had to choose on a scale of 1 to 5 (strongly disagree to strongly agree), for each statement they had to choose the option which best applied to them. We also added four free response questions to understand better about their experience in online learning. The questionnaire was shared among the students with the help of their school academic heads. Participants were of class 9-12 from Luck now Public School and National Public School. A total of 162 students provided complete information with respect to the study. We did Cronbach's alpha test to check the reliability of the questionnaire. Questionnaire has shown to be a valid and reliable measure of the dimensions of depression, anxiety, stress, tiredness, stress and psychological distress among students. **T**hese 26 questions of 5 Point Likert Scale have passed the reliability using Cronbach's Alpha Measure of Internal Consistency.

Ethical Approval

Considering ethical factor, approval to conduct this survey was obtained from the principals of both the schools. Students from class 9-12 voluntarily participated in this survey. The questionnaire was coded to provide discretion and secrecy.

Data Analysis

The quantitative data was entered into EXCEL, then encoded and analysed using descriptive statistics (mean, t-Test: Two-Sample Assuming Equal Variances). The test consists of 26 statements and four open-ended questions that are divided into four categories: despair, anxiety, a sense of prosperity, and general well-being. Because this determining instrument takes into account a variety of variables or facets of psychological well-being, it provides a useful and comprehensive picture of psychological well-being. There are five alternative responses to each of the 26 questions. Although the measuring instrument can be given a total score (which can range from 26 to 130, with a lower score indicating a lower degree of psychological wellbeing), each subscale can also be given a score, which was done in this study.

Findings

The paper brings out student's mental health prosperity during online learning platform. The paper canters around problems experienced by students in virtual schooling set up. The participants were asked questions that concerned the online learning problems, how they felt about teachers were attentive and caring towards them, if they were able to express themselves confidently during online classes, if they were happy during online classes, if they felt sad

when they were not able to match with the pace of class, if they were stressed because of too many assignments in online classes, if they were often irritated in online classes etc.

Psychological Wellbeing of the Students

The students were re-classified into low and high categories based on the total score from 26 statements. A total score can be determined for the measuring instrument (the total score can vary between 26 and 130, with a lower score indicating a lower level of psychological wellbeing). With regard to the responses from students, Figure 2 reveals that the overall psychological wellbeing issue for the majority of the students was "high". Sub-dimension namely positive wellbeing, self-control, anxiety, depressed mood, feeling of taken care of, stress, loneliness. Majority of students fell into 43.7 – 82.2 score, which is relatively high and denotes psychological wellbeing issues among students.**(**Mean, t-Test: Two-Sample Assuming Equal Variances) was calculated in excel to compare the psychological wellness among students of class 9-10 and class 11-12.Since P(T<=t) two tail > 0.05 (significant level), the results indicate no significant statistical difference in the state of psychological wellbeing between both the group during online classes. Class 9-10 and Class 11-12 both groups were falling under high psychological wellbeing issue.

DISCUSSION

The data analysis demonstrated that students associated with this investigation have rather significant degrees of high psychological wellbeing issues. Furthermore, it is seen that the students are having anxiety, stress, depression alongside loneliness state. More significant levels of student's anxiety, stress, depression state of mind stage has likewise been seen in different researches (Hjeltnes *et al.,* 2018; Dewaele J.M., Magdalena A.F., Saito K, 2019; Parray and Kumar, 2017).It was found in the study that there were no significant group differences in class 9-10 and class 11-12 with respect to stress, depression, anxiety, positive prosperity and by and large psychological general prosperity. The tension level was high with all classes, all students were having high psychological wellbeing issues. It very well might be on the grounds that recently confronted social and scholarly difficulties may cause psychological and emotional imbalance, which may prompt an expanded danger for stress, anxiety, depression (Wang *et al.,* 2019).Psychological distress shows the foundation of laziness and boredom in students, brought about by different social conditions. Boredom is likewise brought about by the expanding distance between individuals, because of social distancing and the restriction to step out from the house.

Students are only occupied with themselves to finish their tasks. Depression and anxiety emerge and deteriorates in light of the fact that there is no relational interaction (Furlong, Gilman, and Huebner, 2014). Online learning that causes students to stay in touch through digital devices makes the interaction between one individual and others come up short on non- verbal communication. The shortfall of these communication, combined with limitations on actual in person communication, make students exhausted. Stress, nervousness, depression and anxiety are the normal emotional well-being issues that influence almost two-fifth of the studied populace and in this manner essentially adding to the wellbeing trouble (Wang *et al.,* 2019). Emotional impact on psychological wellbeing, described by moods and mood swings. Student with uneasiness anxiety and stress issues like aggravations in mind-set, thinking, changes in mood or mood swings and behavioural pattern, show a latent mentality in their scholastic activity like absence of interest in study, not getting good grades in assessments and an upset daily schedule. Students experienced aggravations because of an excessive number of assignments, and they thought about that only studying into online setup was not adequate. Students even accept that teachers are not savvy in deciding valuable learning patterns.

Students also grumbled about the absence of social help from their companions. Absence of actual face to face interaction is one issue. Sleep is very important for maintaining the peace of mind and digital devices can influence sleep quality. Sleep plays a vital role in development of brain. Students responded that they had severe sleeping issues because of assignments and had to study more to properly understand the chapter, so they intend to study at night to match the pace of the class which resulted in often waking up often from sleep and created sleep disturbance

and in some cases struggle to sleep even when trying to. Poor sleep quality hampers wellbeing since good sleep allows sharper memory. It is scientifically proven that learning improves with a decent 7-hour sleep at night. Online learning limits face to face communication causes absence of social communication, so verbal and non-verbal communication isn't utilized accurately. Students often grumble about circumstances that trouble them in learning. Absence of social help makes them experience aggravations. Eventually, learning was felt to be less impactful. To diminish aggravations, a few students attempt to interact with teachers and take their help to ask about their doubts when they do not understand some concepts. Results shows that students are having anxiety issues and are upset because of absence of classroom collaboration, examination stress, not able to cope up with syllabus. There are different reasons for issues faced in online Learning like course quality to as expected, the use of content, non-accessibility of specialized help, and less probability of collaboration with peers, and technical difficulties.

CONCLUSION AND RECOMMENDATION

This paper emphasizes the role of online learning on psychological well-being on the students and the academic problems faced by the students residing in Uttar Pradesh, India. Online learning does have its benefits, but it does facilitate anxiety isolation and depressive tendencies in the school going children. The paper discoveries show the psychological well-being of students on online learning. Students are exhausted with online learning. Students experienced a scope of difficulties such as emotional changes for every day schedules, social disengagement, new virtual learning conditions. Depression, anxiety and nervousness among students have been seen. Aggravations are demonstrated by mood swings or disposition brought about by an excessive number of tasks that are considered inadequate by students. Sleep disturbance is also commonly seen among students which hampers wellbeing since good sleep allows sharper memory. To conclude online learning is having various impacts on school going children life and we cannot keep today's school going children away from such platforms however it is critical for parents to understand and enforce safe, responsible use of online learning amongst making them responsible digital citizens in turn. Students' psychological well-being is highly influenced when confronted with sudden shift to online learning, and they need consideration, help, and backing from the society, schools and family. School going children need someone to listen to them and if given an ear, they come up with discussions regarding their deep- most feelings and attitude. Encouraging such healthy discussions between the parent-child duos may lead them to maintain positive psychological well-being among students. It is suggested that the schools ought to team up with parents to tackle this issue by offering psychologist assistance. Therapists and guides can attempt to offer to conquer the impacts of online learning.

Limitations

It was found in the paper that online learning has isolated students concerning pressure, stress, depression, anxiety, positive prosperity and at large psychological well-being. However, this examination explores a critical result, nonetheless, it isn't without limitation. Firstly, target samples were only from class 9-12. Further examination could be led using longitudinal plan in a broader concept among students of all the classes except only class 9-12. Another limitation is the simplicity of the analytical approach. For future researcher, it is recommended that investigation might be directed with a large sample size, and to investigate a solution and identifying strategies to conquer the psychological well-being effect of the online learning on students.

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Table 1: Reliability

Variables	Description	Values	Internal Consistency
K	no. of items	27	
$\sum s^2y$	sum of the item variance	0.068	
s^2x	variance of the total score	133.6	
a	Cronbach's alpha	1.038	Excellent

Table 2: t-Test: Two-Sample Assuming Equal Variances

	Class 9-10	*Class 11-12*
Mean	64.857143	64.22222
Variance	115.70507	161.3787
Observations	63	99
Pooled Variance	143.68016	
Hypothesized Mean Difference	0	
Df	160	
t Stat	0.3286634	
P(T<=t) one-tail	0.3714199	
t Critical one-tail	1.6544329	
P(T<=t) two-tail	0.7428398	
t Critical two-tail	1.9749016	

Figure 1: Psychological Wellbeing Score

RESEARCH ARTICLE

Effect of Core Training and Circuit Training on Selected Physical and Physiological Variables among College Level Cricket Players

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ABSTRACT

Core training is the strengthening and conditioning of the core muscles surrounding the middle of the body the abdomen, hips, pelvis, and lower back. These muscles protect the spine and are responsible for stabilizing and balancing the body during movement. Therefore core training is sometimes referred to as core stabilization or balance training. Strong core muscles that contract appropriately are important for good posture and balance, and for the stability and mobility of the spine, rib cage, pelvis, and hips. Movement is more powerful and efficient with a strong core. Strong core muscles also give better definition to the superficial muscles of the trunk and can help prevent or reduce lower back pain and injuries. The purpose of the study was to find out the effect of core training and circuit training on selected physical and physiological variables among College Level Cricket players. To achieve this purpose, forty five male Cricket players were selected as subjects, their aged between 18 to 25 years, they are studying in the affiliated Arts Colleges, Coimbatore, Tamil Nadu. The selected subjects were divided into three equal groups of fifteen subjects each, namely core training group, circuit training group and control group. The core and circuit training group trained for three sets per exercise per session at 60 to 80% with a progressive increase in load with the number of weeks. speed and Resting heart were selected as criterion variables and they were tested by using 50 mts dash and pulse test respectively. ANCOVA was used to find out the significant difference if any between the groups. The results of the

study showed that there was a significant difference on speed and resting heart rate between core training and circuit training.

Keywords: Core training , Circuit training , speed and Resting heart rate

INTRODUCTION

Core training is the strengthening and conditioning of the core muscles surrounding the middle of the body the abdomen, hips, pelvis, and lower back. These muscles protect the spine and are responsible for stabilizing and balancing the body during movement. Therefore core training is sometimes referred to as core stabilization or balance training. Strong core muscles that contract appropriately are important for good posture and balance, and for the stability and mobility of the spine, rib cage, pelvis, and hips. Movement is more powerful and efficient with a strong core. Strong core muscles also give better definition to the superficial muscles of the trunk and can help prevent or reduce lower back pain and injuries. In Cricket, it can be played more skillfully when players have the power that combines with strength and speed to develop explosive power for participating in various sports activities. The core exercises and circuit training improve significantly in developing physical and physiological variables among Cricket players.

METHODOLOGY

The purpose of the study was to find out the effect of core training and circuit training on selected physical and physiological variables among cricket players. To achieve this, thirty male Cricket players are studying in the affiliated Arts Colleges, Coimbatore, Tamilnadu in the age group of 18 to 25 years were selected as subjects at random. The selected subjects were divided into three equal groups of fifteen subjects each namely core training group , circuit training and control group. The selected criterion variables such as speed and heart rate were assessed using standard tests and procedures, before (pre test) and after (post test) training Regimen for both experimental and control groups by using sit-ups and shuttle run respectively. The selected subjects had undergone the core training for eight weeks, with three days per week in alternate days. After 10 to 15 minutes of warm-up the subjects underwent their respective core training programme and the subjects performed core exercises. The control group did not participate in any specialized training during the period of study.

RESULT AND DISCUSSION

The experimental design used for the present investigation was random group design involving 30 subjects for training effect. Analysis of Covariance (ANCOVA) was used as a statistical technique to determine the significant difference, if any, existing between pretest and post test data on selected dependent variables separately and presented in Table-I.

RESULTS OF SPEED

Table I reveals that the F-value for pre-test 0.15 and post-test 13.51 among the experimental groups (core strength training group and circuit training group) and control group on speed. The obtained F-ratio for pre-test and post-test to be significant at 0.05 level for degree of freedom 2, 57 the required critical value was 3.16.hence, the F-ratio (0.15) obtained for pre-test was found to be not significant since it do not reach the required critical value 3.16.Regarding the F-ratio for post-test mean (13.51) was found to statistically significant since it was higher than their required critical value 3.16. Based on F-ratio it was informed that experimental group and control group are equal in this performance of speed before they included into their respective treatment whereas, after completion of 12 week treatment period, experimental groups and control group were significantly different from one another in the performance of speed. The F-ratio for speed (28.18) obtained for adjusted post test mean was found to be

significant at 0.05 level for degree of freedom 2, 56 the required critical value was 3.16. Based on the results, that the significant difference among the effects of training namely core strength training group, circuit training group and control group on physical fitness variables and physiological variables among cricket players. The mean value of speed among core strength training group, circuit training group and control group are graphically represented in figure 1.To identify the specific differences among the three groups Scheffe's post hoc test was used. The results of post hoc testes are presented in table II .Table II shows the adjusted post-test mean of core strength training group, circuit training group and control group on speed. The obtained mean difference between the core strength training group and circuit training group, core strength training group and control group, circuit training group and control group were 0.32, 0.57 and 0.25 respectively. The required confidence interval value was 0.12. Since the obtained mean differences between the core strength training group and control group and between circuit training group and control group were greater than the obtained confidence interval value on speed, it was concluded that there were significant differences in the effects on speed. Further the obtained mean differences between core strength training and circuit training was greater than the obtained confidence interval value on speed, hence it was concluded that there was significant differences in the effect of speed.

RESULTS OF RESTING HEART RATE

Table III reveals that the F-value for pre-test mean (1.93) and post-test mean(97.78) among the experimental groups (core strength training group, circuit training group) and control group on Resting heart rate. The obtained F-ratio for pre-test mean (1.93) and post-test mean (97.78) to be significant at 0.05 level for degree of freedom 2, 57 the required critical value was 3.16.Hence, the F-ratio (1.94) obtained for pre-test mean was found to be significant since it do not reach the required critical value 3.16.Regarding this F-ratio for post-test mean (97.78) was found to statistically significant since it was higher than their required critical value 3.16 Based on F-ratio it was informed that experimental group and control group are equal in this performance of resting heart rate before they included into their respective treatment whereas, after completion of 12-week treatment period, experimental group as control group were significantly different from one another in the performance of Resting heart rate. The F-ratio for resting heart rate (113.01) obtained for adjusted post-test was found to be significant. To be significant at 0.05 level for degree of freedom 2, 56 the required critical value was 3.16. Based on the results, that the significant difference among the effects of training namely core strength training group, circuit training group and control group on physical fitness variables and physiological variables among cricket players.. The mean value of resting heart rate among core strength training group, circuit training group and control group are graphically represented in figure 2 To identify the specific differences among the three groups Scheffe's post hoc test was used. The results of post hoc testes are presented in table IV. Table IV shows the adjusted post-test mean of core strength training group, circuit training group and control group on resting heart rate. The obtained mean difference between the core strength training group and circuit training group, core strength training group and control group, circuit training group and control group were 0.83, 4.39 and 5.22 respectively. The required confidence interval value was 4.84. Since the obtained mean differences between the core strength training group and control group and between circuit group and control group were greater than the obtained confidence interval value on resting heart rate, it was concluded that there were significant differences in the effects on resting heart rate further the obtained mean differences between core strength training and circuit training was lesser than the obtained confidence interval value on resting heart rate, hence it was concluded that there was significant differences in the effect of resting heart rate.

SUMMARY

Cricket is a sport a number of distinct methods of core strength training and circuit training . To succeed as a cricket player in elite competition, the athlete must develop a wide range of physical skills. The ideal cricket player is of tent all and physically very limber. All players, irrespective of their height, will be agile, possessive of speed and resting heart. To achieve the purpose of the present study, 120 players were selected as samples from they are studying in the Affiliated Arts Colleges, Coimbatore, Tamil Nadu. Finally 60 male cricket players were randomly selected as subjects for the present study. They were divided into three groups, each consists of 20 subjects. Group I was named core strength Training (COST), Group II was named circuit Training group (CITG) Group III and control group (CG).The training programme was given for all these three groups for 12 weeks.

Before initiating the training programme the pre-test was taken on the selected variables. The readings on variables were recorded carefully in their respective units. The collected data were treated with both univariate and multivariate statistical analysis to test objective of the present study.

CONCLUSION

The pre-test before the related training showed that there was an insignificant and variation on speed and Resting Heart Rate among the three groups. The post-test after the related training showed significant improvement on speed and Resting Heart Rate. In the core strength training group and in the circuit training group than the control group. Comparisons among these three groups resulted that the core strength training group shows better improvement in all the selected variables than the circuit training group and control group. The result also revealed that the speed and resting heart rate were comparative better in the core strength training group than the circuit training group after the related training.

RECOMMENDATIONS

Based on the results of the study, the following recommendations have been made.

1. In the physical exercise, while designing the training programme the effect of varied training modalities is explained positively on muscle fitness parameters and skill performance variables of cricket players, the physical education teachers, trainers and coaches can prefer this type of training so as to achieve their aim in time.

2. Training with core preceded with circuit training enhances the selected muscle fitness parameters, physiological and skill performance variables of cricket players. This is due to integrating the core with circuit training which requires the players to perform the core exercises in a fatigue stage, resulting in potentially increasing explosive power production. Hence the volleyball players can use this type of training as a module in order to achieve high level skill performance in the game of cricket .

3. In a combined training routine, a player performs a heavy set of traditional core training exercise, which is followed almost immediately by a circuit exercise. In this study this type of combination proves to be effective in developing the fitness variables and skill performance, the coaches can utilize this training strategy for better performance of the players.

4. Another training strategy is known as complex training in which a player alternates biomechanically similar high load resistance training exercises with core exercises, set forest, in the same workout. Since this type of training also proves to be effective in developing the fitness parameters and skill performance of the cricket players the coaches can utilize this technique in their conditioning programme to develop the fitness and skill performance.

DISCUSSION OF FINDINGS

The pre-test before the related training showed that there was an insignificant and variation on strength endurance and agility among the groups. The post-test after the related training showed significant improvement speed and resting heart rate . In the core group and control group. Comparisons among these two groups resulted that the core training group shows better improvement in all the selected variables than the control group The result also revealed that speed and resting heart rate were comparative better in the control group after the related training.

RECOMMENDATIONS

Based on the results of the study, the following recommendations have been made. In the framing of training while designing the training programme the effect of varied core training programme is explained positively and physical fitness variables of cricket players. This is due to integrating the core training which requires the players to perform the exercises in a fatigue stage, resulting in potentially increasing endurance. Hence the cricket players can use this type of training as a module in order to achieve high level skill performance in the game of cricket.

Based on the results of the study, it was concluded that the core and strength training program has resulted in significant increase in selected physical fitness variables such as speed and resting heart rate.

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Table 1. Analysis of covariance among the core training circuit training and control group on speed

	Group	Mean	Source	Sum of Square	df	Mean Square	F-ratio
Pre-test	COTG	6.95	B/S	0.06	2	0.03	0.15
	CITG	7.02	W/S	11.57	57	0.20	
	CG	6.96					
Post test	COTG	6.38	B/S	3.37	2	1.68	13.51*
	CITG	6.73	W/S	7.10	57	0.12	
	CG	6.95					
Adjusted Mean	COTG	6.39	B/S	3.26	2	1.63	28.18*
	CITG	6.71	W/S	3.24	56	0.06	
	CG	6.96					

Table 2. The scheffe's post hoc test on speed

Variables	COTG	CITG	CG	MD	CI
Speed	6.39	6.71	------	0.32*	0.12
	6.39	-------	6.96	0.57*	
	------	6.71	6.96	0.25*	

Table 3. Analysis Of Covariance Among The Core Strength Training Circuit Training And Control Group On Resting Heart Rate

	Group	Mean	Source	Sum of Square	Df	Mean Square	F-ratio
Pre-test	COTG	73.10	B/S	14.23	2.00	7.12	1.93
	CITG	72.25	W/S	210.35	57.00	3.69	
	CG	73.40					
Post test	COTG	69.30	B/S	356.63	2.00	178.32	97.78*
	CITG	68.15	W/S	103.95	57.00	1.82	
	CG	73.80					
Adjusted Mean	COTG	69.23	B/S	302.07	2.00	151.03	113.01*
	CITG	68.40	W/S	74.84	56.00	1.34	
	CG	73.62					

Table -Iv The Scheffe's Post Hoc Test On Resting Heart Rate

Variables	COTG	CITG	CG	MD	CI
Resting Heart rate	69.23	68.40	------	0.83	4.84
	69.23	-------	73.62	4.39	
	------	68.40	73.62	5.22*	

Figure 1. The mean values of pre-test post-test and adjusted mean of core strength training group circuit training group and control group on speed

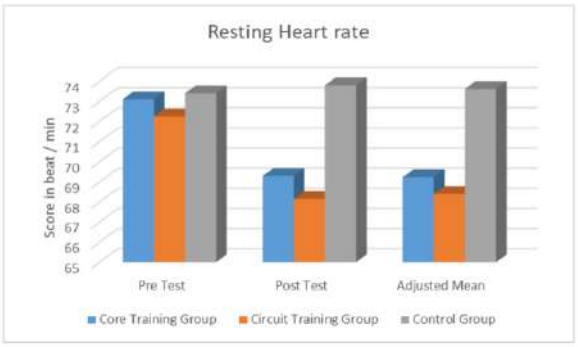

Figure 2. The mean values of pre-test post-test and adjusted mean of core strength training group circuit training group and control group on resting heart rate

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RESEARCH ARTICLE

The Role of Resonance in Physical and Biological Nonlinear Systems

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ABSTRACT

For a class of physical and biological nonlinear systems, the concepts of resonance, forms of resonance, and applications of resonance are proposed. Resonances and resonant frequencies are also analysed in terms of damping. These findings offer a new perspective on energy transfer in nonlinear systems and demonstrate how damping affects the system's resonant frequencies and amplitudes.

Keywords: physical and biological, Resonances, nonlinear systems

INTRODUCTION

Originally, the term resonance (from Latin resonantia 'echo,' from reson are "resound") was coined to describe the acoustic phenomenon of strings vibrating and producing sound without the player's direct excitation. This discovery was made in the year 1739 by Swiss mathematician and physicist Leonhard Euler (1707–1783), who discovered resonance by solving an ordinary differential equation. The Italo-French mathematician Joseph Louis Lagrange (1736–1813) began his study of resonance by working with sound and music in an extremely cautious manner. Resonance was an important topic for German physicist Hermann von Helmholtz (1821–1894), who wrote extensively on the subject of sound waves and the mechanics of the human ear. Special note should be made of his book On the Sensations of Tone as an Anatomy of the Theory of Music, where the quadratic nonlinearity of a notional system first appeared. Resonance has been observed in a wide variety of systems, ranging from biological to chemical and physical systems, as well as mechanical and engineering systems. The study of resonances is important in many fields of biology. Connected by resonance, cells and bodies can transmit energy across domains, resulting in a constant state of interaction and interference that keeps the entire cosmos connected. Consciousness has always been associated with the nervous system, or more specifically the brain, but recorded conscious behaviours in

organisms lacking nerve cells have altered our understanding of consciousness. A living cell is a blend of resonant frequencies that vibrate as a harmonic oscillator, supporting the progression of vibrations as waves in and out of the system; to neighbouring cells, the body, other bodies, and ultimately the Universe; all of which connect. Quantum generated consciousness is energy; it emerges in every living cell and travels through resonating vibrations, utilising the cellular structures to flow in a coherent, obscured, robust, and recoverable process. In linear system analysis, resonance is a well-known concept. At a resonance, the frequency of an exciting force matches the natural frequency of the system, resulting in efficient energy transmission and significant vibration amplitude. It refers to the achievement of a system's maximum response. The response of an oscillating system is primarily determined by the system's ability to store and transfer energy received from an external forcing source into an internal oscillating mode. Resonance can occur in both microscopic and macroscopic systems, and it can be deterministic or stochastic in nature. Resonant behaviour can be seen in both single and coupled systems. Many applications benefit from it, but others suffer from instability and disasters as a result. External factors can cause resonance in systems. Resonances can be generated by a variety of external factors. An external periodic force increases the maximum responsiveness of a system at a certain frequency, and this is known as a forced resonance or simply a resonance. In order to get the systems to behave the way you want them to, it's critical that you have a firm grasp on how resonances work. Linear systems are assumed in nearly all investigations of resonance. Nonlinear components of biological systems, on the other hand, cannot be adequately captured by a linear model. There are many examples of resonance in the cardiovascular system (CVS): (i) myogenic oscillations (myocardial oscillations) at 0.1 Hz; (ii) respiratory oscillations (respiration) at 0.2; (iii) neurogenic processes and VT baroreflexes at 0.03 Hz; (iv) NO-dependent and NO-independent endothelial oscillations near 0.01 Hz that provide an indication of endothelial health on which the immune system relies; (v) endothelial oscillations (endothelial oscillations) near 0.01 Hz that provide an indication of endothelial health on which the immune. Due to the fact that these oscillations are part of a tightly coupled system, they all influence each other. Many physiological variables, such as blood pressure and blood flow, reflect their presence, and all of them have the potential to be useful in clinical studies. In nature, nonlinearities play an important role in a wide range of processes. Nonlinear phenomena can be understood and mastered with a thorough understanding of the laws of nonlinear science. Researchers in the domains of mathematics, physics, chemistry, biology and epidemiology have invested in these nonlinear sciences in order to achieve this goal.

Owing to its wide range of applications, nonlinear science has garnered worldwide attention. These include the physical, technological, biological, and social sciences, as well as the humanities. Nonlinearity is a fundamental property of many natural and technological systems, and it influences their behaviour. Nonlinearity isn't the only thing that can affect these systems; stochastic/noisy or deterministic/periodic driving forces have been documented as well. Resonance, a phenomenon in physics originally linked to the matching of a system's natural frequency of vibration to the frequency of an external force driving it, or the matching of two or more frequencies within a system, and ultimately leading to an enhancement of the output signal, can be induced by the action of driving forces on nonlinear systems. Resonance can occur in a variety of ways in most systems, and it isn't always tied to frequency matching as previously thought. So the definition of resonance was expanded to include all known processes that lead to the enhancement, suppression or optimization of a system's response through variation/ perturbation /modulation of any system property, thus removing the restriction. There are many examples of nonlinear systems in nature. Their dynamics has been studied extensively, in part because of their wide range of applications, ranging from the physical and mathematical sciences to engineering and the life sciences. Among the many interesting and important properties that nonlinear systems can exhibit when driven by external forces is resonance.

Nonlinear Resonances of various kinds

A nonlinear system's maximum response is strongly dependent on the properties of the external driving force, which is called nonlinear resonance. Oscillatory systems' instability and multistability are largely due to nonlinear resonance. Nonlinear resonance is typically only taken into account when a single frequency is being forced.

(a) The first is **stochastic resonance**, which is triggered by a weak noise at the frequency of the periodic force applied.

(b) A high-frequency force at the low-frequency of the external forces causes **vibrational resonance.**

(c) When there is no periodic force external to the system, a **coherence resonance** is produced.
(d) **Resonance** that occurs when an external force has a frequency that is not present in the multi-frequency force.
(e) A periodic variation in a parameter of the system causes **parametric resonance**.
(f) Resonance induced by an external force with time-dependent frequency is known as **auto resonance.**
(g) A **chaotic resonance** occurs when the natural order is disrupted.

There are two types of additive resonance that occur in both linear and nonlinear systems: parametric resonance and additive resonance with a single frequency. Nonlinear systems are the only places where other resonances can occur. In addition to the above resonant frequencies and control parameter values, the response amplitude is found to be zero or minimum in certain systems at one or more frequencies. Antiresonance is the name given to this phenomenon. In mathematical model equations of physically interesting systems and in real experimental systems, the salient features of the aforementioned resonances and antiresonances have been studied. In order to identify and investigate the various characteristics of resonances, theoretical procedures and statistical measures are developed.

Examples of Resonance in Simple Forms

In a playground, a swing's oscillatory motion is an excellent example of resonance. Stable equilibrium positions are found at rest. A mother is pushing her child on a swing. A good place to start is by releasing the swing's seat back. Swinging back to the starting position after reaching equilibrium is a common occurrence. Its maximum speed is reached when it reaches equilibrium. As soon as the speed drops to zero on the other side, the swing begins to reverse. A decrease in swing amplitude due to chain friction against the bar support would eventually bring it to an end if the mother doesn't give any more pushes. The mother must push the swing at the right time in order for it to keep going back and forth without a pause.. When the swing is at its peak amplitude, it is the best time to make a decision. Pushing from mom and swing motion are in perfect sync at this precise moment. Even as a child, Galileo Galilei (1564–1642) observed that a single man alone by giving impulses at the right instant was able to ring an enormous bell that when four or six men seized the rope and tried to stop it they were lifted from the ground, all of them together being unable to counterbalance the momentum that a single man, by properly timed pulls, had given it. This is still used in the ringing of heavy free-swinging tower bells.

Nonlinear and Harmonic Resonances

A single particle or a group of particles can make up a physical system. The term "oscillator" refers to a system that oscillates back and forth. A wall-clock pendulum oscillator is a well-known example of an oscillator. For example, an oscillator can be periodic, quasiperiodic, or even chaotic (which is bounded non periodic movement of nonlinear system with high sensitive dependence on initial conditions) in its oscillations. As long as the system is not influenced by any external forces, oscillations are referred to as "natural" or "free." Energy dissipation reduces the frequency of natural fluctuations and brings the system to a steady state known as an equilibrium position over an extended period of time. The term "forced oscillations" refers to oscillations that are induced by an external periodic force. Forcing an oscillation to stabilise after an initial burst of movement is common. Periodic driving force period is an integral multiple of the periodic oscillatory force period. Let's say that we increase by a factor of two the frequency of the external periodic force. When the frequency is increased further, the amplitude of the oscillation decreases, but only if the frequency is increased to a certain value. This is typical. Resonance is a term used to describe this phenomenon in which a peak in amplitude occurs. The angular frequency of a driving force can be varied to achieve a high amplitude in a pendulum, for example. Resonant frequency is the frequency at which the amplitude of oscillation reaches its peak (Fig. 1). Generally speaking, the resonant frequency of a linear system with weak damping is approximately equal to the system's natural frequency. Small periodic driving forces can produce large oscillations at the resonant frequency, even if their magnitude is small. Linear and nonlinear systems both exhibit resonance. Linear and nonlinear resonances share some characteristics, but they also differ in important ways. Many fields of physics, engineering, and biology make use of resonance. Devices such as swing sets and quartz watches are examples of mechanical resonance. Other examples include the acoustic resonance in musical instruments and human vocal cords and the electrical resonance in televisions and radio sets (creation of coherent light in a laser cavity). Because of internal vibrations, some objects have a resonant quality (called resonators). Quartz

crystals, vibrating strings and laser rods are just a few examples. Nerve cells and heart cells are the two most prominent examples of excitatory systems. Neurons are essentially information processing units. This feature requires a special mechanism to reliably detect very weak signals in complex situations. Therefore, it is important to investigate the potential for vibrational resonance in excitatory systems and the corresponding mechanisms. Motivated by the above, Ulllner and his collaborators examined Chua diodes and electronic circuits based on the FitzHugh-Nagumo (FHN) model equation showing excitatory dynamics, and high-frequency drive signals in amplitude. Demonstrated the optimum amplitude of. Low frequency boost. Oscillating resonances in networks of FHN equations have also been studied. The FHN model was created by American biophysicist Richard FitzHugh (1922–2007), who proposed the system in 1961, and Japan's Junichi Nagumo (1926–1999), who developed an equivalent circuit in collaboration with Arimoto the following year. It was named after it. Yoshizawa describes the firing activity of sensory neurons. The FHN model equations are given by Keener and Snyder and Mikhailov.

Resonance-based applications

In radio, television, and playground swings, resonance is critical. Vibrations of a specific frequency can be generated, and frequencies from a complex oscillation containing multiple frequencies can be selectively selected. Rare gas and metal clusters driven by near-infrared lasers benefit greatly from ionisation of the outer layer and energy absorption. Nonlinear resonance was used in epidemiology to explain periodic cycles of measles, childhood diseases, wild life diseases, and species invasion properties. Following is a list of notable resonance applications.1. The five senses of sight, sound, touch, and smell are all stimulated by vibration and resonance in the human body (smell). An essential principle of vibrational therapy/medicine is that all matter vibrates at a specific wavelength and, by using a resonant vibration, the balance of matter can be restored.2. Patient's gall stones are broken up by ultrasound resonance. 3. The radio tuning device is a well-known example of resonance in action. Each radio station uses a unique frequency to broadcast. A radio's receiving circuit is subjected to a periodic jolt from all broadcast waves. The tuning knob adjusts the capacitance or inductance of the circuit. It's possible to hear a particular station's signal when its frequency falls within the resonant frequency range of the circuit. All other signals, however, remain below the audible level. In the same way, a radio telescope's receiver can be fine-tuned to pick up signals at or near a specific wavelength. 4. Harmonics of the fundamental (strongest) resonance frequency can be vibrated by a number of resonant objects. Many clocks use a pendulum or balance wheel to keep time. 5. Nonlinear resonances are responsible for the helium dielectric permittivity variations in superconductors. 6. Certain nano-electromechanical systems (NEMS) have emerged as successful memory devices. "If resonance is present, the device is classified as static. If resonance is absent, the device is classified as dynamic. The nonlinear regime of operation is unavoidable in NEMS. There is a strong correlation between the oscillation amplitude and the resonance frequency in this regime. The amplitude fluctuation has the unintended consequence of causing frequency fluctuations. In the nonlinear regime, this is considered a major drawback. Experimentally, the stability of oscillation frequency in NEMS is achieved by coupling two different vibrational modes through an internal resonance. A resonance in one mode of vibration absorbs most of the fluctuation in the other's frequency and amplitude due to the energy exchange between the two modes. 7. Nonlinear resonant responses of biological tissue to electromagnetic fields are used to investigate cancer cases. 8. It is called nuclear magnetic resonance when electromagnetic radiation is absorbed and re-emitted by nuclei in the presence of an external magnetic field (NMR). A substance's resonance frequency determines how much energy it absorbs and how much it emits. It is the strength of the applied field as well as the magnetic properties of the atom isotope that determine the resonance frequency. Magnetic resonance imaging makes use of this property in studying crystals, non-crystalline materials, and molecular physics with NMR spectroscopy (MRI).

CONCLUSION

As a result of this article's research, a wide range of disciplines, such as neuroscience, communication, image processing, and medicine were able to utilise the concept of resonance in driven systems. Resonance and resonant frequencies are well-known concepts in linear systems, but there are no equivalent concepts for nonlinear systems, despite the fact that they have been observed in nonlinear systems. Resonance behavior involving mechanical

vibration of system elements can be expected in many biological systems. They tend to have microwave frequencies as their natural frequencies. Microwave exposure may have physiological effects on humans and other species because some of these systems will be electrically coupled to the electromagnetic field. Such microwave excitable resonances, on the other hand, are expected to be strongly dampened by their aqueous biological environment. There has been research into those energy dissipation mechanisms but little attention has been paid to how the limited coupling of these resonances to the electromagnetic field limits energy transfer. If the coupling is very small, the absorbed energy is so strongly limited that resonances cannot affect biology significantly even if the systems are much less strongly damped than expected from the basic dissipation models. As a result, one of the goals of this article is to provide a platform for the exchange of ideas between experts in various fields.Resonance properties are expected to lead to advanced technological applications in the near future. It has been discovered that many macro, micro, and nanoscale oscillators and devices that operate in resonant modes as filters, nonlinear mixers, sensors, and atomic scale imaging and amplifiers improve efficiency and performance dramatically.

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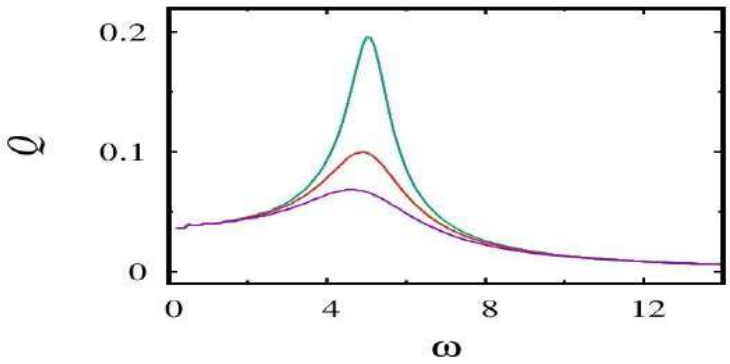

Figure 1: Frequency response - Resonance curve

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RESEARCH ARTICLE

Studies on Effect of Seed Priming Treatments, Containers and Period of Storage on Seed Quality in Barnyard Millet (*Echinochloa frumentacea*) cv. CO 1

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ABSTRACT

The present investigation 'studies on effect of various seed priming treatments, containers and period of storage on seed quality in barnyard millet cv. CO 1' involves laboratory experiments were carried out at Seed Science and Technology Laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram. The seeds of barnyard millet cv. CO 1 were given with following seed treatments i.e., dry dressing with Thiram @ 2g/kg, Hydropriming, bio priming with Pungam Leaf extract @ 5%, Moringa Leaf extract @ 5%, organo priming with Vermiwash @ 5%, Panchagavya @ 5%, halo priming with $MnSO_4$ @ 2%, $ZnSO_4$ @ 2% along with control. The effect of seed priming treatments and containers on the storability of barnyard millet was studied at ambient condition of Annamalai Nagar. The barnyard millet cv. CO 1 primed with 5% moringa leaf extract for 6 hrs and stored with aluminium container seeds registered high germination percentage, root length, shoot length, dry matter production and vigour index when compared to control. This type of seed storage in barnyard millet cv. CO 1 maintained the minimum seed certification standard till the end of the storage period.

Keywords: Seed priming, Germination Percentage and Vigour Index

INTRODUCTION

Barnyard millet (*Echinochloa frumentacea* L.) also called as sawa millet. It is frequently cultivated in India, China and Niger, Nigeria, also in Mali, Burkina Faso and Sudan. Because of its low carbohydrate content and slow digestion, barnyard millet is a nature's gift to the sedentary modern man. Low in phytic acid and also barnyard millet grains are enriched in calcium and iron [1]. Seed-enhancement technologies have a primary goal of enhancing seed performance by using specified additives (chemical, organic, or botanical) and planting equipment in order to cultivate a steady crop, which in turn leads to increased yields and productivity [2]. As a pre-sowing seed treatment, seed priming has been proved to have an invigorative impact. Priming is an effective method to improve the seed's germination rate and uniformity. Variety of plant species benefit from seed priming, a procedure that enhances seed quality, increases germination rates, and prolongs seed storage. Seed quality is determined by genetic make-up, although the quality of seeds frequently deteriorates during storage. Seed vigour is highly influenced by poor storage conditions. As a result, maintaining seed viability and vigour requires proper regulation of seed moisture content as well as seed storage conditions. Packaging is important for storage as well as protection from environmental conditions, mechanical and physical risks during storage, transportation, and marketing [3]. The type of packing material to be used is determined by a number of inter-dependent factors, including the type and quantity of seed handled, the type and size of packs, the targeted storage duration, the storage environment, and the geographic area of storage[4]. Seed quality maintenance throughout storage is important for crop production as well as preservation of seed integrity due to the ongoing threat of genetic erosion. Keeping these above view, the present investigation undertaken in barnyard millet cv. CO 1 with the following objective "To make a comparative assessment of various seed priming treatments, containers and period of storage on seed quality in barnyard millet cv. CO 1."

MATERIALS AND METHODS

Genetically pure seeds of barnyard millet (*Echinochloa frumentacea*) cv. CO 1 obtained from Centre of Excellence for Millets, at Athiyandal in Thiruvannamalai served as the base material for the study. The Laboratory experiments were conducted at Seed Science and Technology Laboratory, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India (located at 11˚ 24'N latitude and 79°44'E longitude with an altitude of + 5.79 mts above mean sea level) during 2019 – 2021.Bulk seeds of barnyard millet cv. CO 1 were cleaned and graded. Then the seeds were given with following seed treatments.

Treatment details

T_0- Unprimed(Control)
T_1- Dry dressing with Thiram @ 2g/kg
T_2- Hydropriming
T_3- Bio priming with Pungam Leaf Extract @ 5%
T_4– Bio priming with Moringa Leaf Extract @ 5%
T_5– Organo priming with Vermiwash @ 5%
T_6– Organo priming with Panchagavya @5%
T_7– Halo priming with Manganese sulphate @ 2%
T_8- Halo priming with Zinc sulphate @ 2%

For hydro priming, seeds were soaked in equal volume of water for 6 h and shade dried to original moisture content. For Halo priming, the nutrient solutions of 2% MnSO4 and 2% ZnSO4 were prepared by dissolving respective 2 g salt in to 100 ml distilled water, in which barnyard millet seeds were soaked for 6 h. After priming, the seeds were removed from the solutions and shade dried. For Bio priming, the fresh leaves of Pungam (*Pongamia pinnata*), Moringa (*Moringa oleifera*) were collected separately and dried under shade. The shade dried leaves were

powdered using mortar and pestle. Then exactly weigh five gram of leaf powder using weighing balance and dissolved in 100 ml of distilled water which was measured already in the beaker to make 5% leaf extract. The leaf extract was filtered by using muslin cloth to remove unwanted materials and leaf debris. The seeds were soaked in equal volume of (1:1) leaf extract for 6 hours along with water. The soaked seeds were dried back to original moisture content. For organo priming, the seeds of barnyard millet cv. CO 1 added with 5% vermin compost extract and 5% panchagavya at 1:1 ratio for 6 hrs. After priming for 6 hrs, the seeds are removed from the solution, rinsed in distilled water, shade dried in room temperature and the unprimed seeds are used as control. The above treated seeds were assess quality characters in seed storage. Storage trial was conducted by adopting FCRD with three replications. Then the treated seeds were dried to the moisture content below 12.0 per cent were packed in cloth bag and 8.0 per cent were packed in Aluminium container and stored under ambient conditions. The following physiological seed quality determinations were made initially and subsequently once in two months interval.viz., germination percentage, root length, shoot length, dry matter production and vigour index when tested for variation among the seed priming treatment, container and period during storage in barnyard millet cv. CO 1.The data pertaining to the observations in the laboratory experiments were statistically analysed adopting the procedure described by [5]. Wherever necessary, suitable transformation was made before analysis.

RESULTS AND DISCUSSION

Millets play an active role in feeding the world's ever-expanding population. Generally small millets are low in phytic acid content and high in iron, dietary fibre, vitamin B and calcium [6]. Seed is a biological entity, deterioration in quality is predictable, persistent, and inexorable. It happens when ageing, which is usual that all living organisms go through. A range of biotic and abiotic factors influence seed storage potential, resulting in seed deterioration and, eventually, seed death. Keeping high-quality seed until it is needed for the next planting is a crucial responsibility for every seed production programme. Seed must be stored safely since it is harvested in the previous season and is normally utilised for sowing in the following season, frequently after a six-month or longer interval. The majority of seed quality deteriorates during storage. To maintain the seeds in good condition, adequate storage is essential. The majority of farmers have no idea how to conserve seed. They store their seeds in the same way as they keep their food grain. Although genetic make-up governs seed quality, the quality of seeds frequently deteriorates during storage. Seed storage and seed viability retention are always critical attributes in agricultural production. Seed vigour is highly influenced by poor storage conditions[7]. High temperature, relative humidity, and moisture in the storage environment constitute the main factors involved in seed quality deterioration. As a result, seed quality has been proven to influence the seed health [8]. The essential prerequisite for seed is germination potential. The two most significant aspects of seed quality are viability and vigour, which go hand in hand when determining seed quality. In the present study, the germination percentage decreased with increase in the storage period *viz*. 92 to 77 per cent (Table 1). The study highlighted that barnyard millet seeds primed with moringa leaf extract @ 5% for 6 h and stored in Aluminum container maintained their germination for minimum seed certification purpose till the end of the storage period. Where the actual germination percent recorded after storage was 88 per cent. The observed findings showed that Moringa, a natural plant hormone that belongs to the cytokinine group, which is involved in increasing the germination percentage [9].When compared to an untreated control, the use of moringa leaf extract significantly improved seed germination. Moringa leaves are high in zeatin, ascorbic acid, calcium, and potassium [10], which promotes seed germination and seedling. The higher concentration of Ca and other mineral content of Moringa leaves may be responsible for increasing seed emergence rate [11]. The results are in conformity with the findings of [12] in sorghum, [13] in ragi, and [14] in maize.

Seed deterioration, as evidenced by loss of viability, which is related to reduced root and shoot development [15]. The root length might be a suitable measure for determining seed vigour. In the present study, the root and shoot length of the seedling showed significant reduction over periods of storage, irrespective of the treatment and container. In the present study, the root length and shoot length decreased with increase in the storage

period *viz*. 12.94 to 10.76 cm for root length and 8.24 to 6.92 cm for shoot length (Table 2 and 3). The study highlighted that barnyard millet seeds primed with moringa leaf extract @ 5% for 6 h and stored in Aluminum container produced lengthier seedlings compared to those stored in cloth bag. At the end of the storage period the above treatment were superior in producing lengthier seedlings than the untreated ones. It produces 12.78 cm root and 7.92 cm shoot. The observed findings showed that moringa leaf extract include major and minor nutrients, amino acids, vitamins, and growth substances such as cytokinins, auxins, and abscisic acid (ABA). It is well renowned for its extraordinary nutritional and medicinal benefits. Tree leaves are high in vitamins (A, B, C), minerals (K, Ca, Fe), antioxidants (Ascorbate, Phenolics), proteins, and the growth hormone zeatin [10]. To ensure optimal shoot growth, the seed primed with MLE takes proper regulation of cytokinin levels inside the plant [16]. These findings were comparable to those of [17], who observed that MLE treated maize has increased root and shoot growth, [18] in okra, [12] in sorghum and [13] in ragi.

The ultimate indication of physiological vigour is seedlings dry matter production. Seedling vigour is often defined by the seedlings weight following a period of growth, and this is an important physiological phenomena mediated by reserve metabolites, enzyme activity, and growth regulators. The vigour estimations based on physiological representations such as seedling length, dry matter accumulation, and the vigour index calculated by multiplying the germination percentage by the seedling length had clearly demonstrated the value of such estimations for determining the seed vigour in storage [19]. In the present study, the dry matter production and vigour index decreased with increase in the storage period *viz*. 0.024 to 0.017 g for dry matter production and 1945 to 1377 for vigour index (Table 4 and 5).. The decrease was low in barnyard millet seeds primed with moringa leaf extract @ 5% for 6 h and stored in Aluminum container. At the end of the storage period the above treatment recorded dry matter production (0.025 g) and vigour index (1823). The observed findings showed that effects of moringa leaf extract on plant height and plant dry weight were substantial. The crop performance improved when primed with moringa leaf extract, particularly during the vegetative and reproductive stages, due to higher metabolic activity at low water potential [20]. The presence of increased zeatin in moringa leaf extract was responsible for the crop's effective growth during the vegetative and reproductive stages [21]. The leaves of *Moringa oleifera* may be responsible for increasing the rate of seed emergence and vigour of the plant. It was attributed to increased mobilisation of inorganic solutes to germinating shoot, which resulted in increased growth [22]. MLE, which is high in K, Ca, Fe, amino acids, ascorbates, and growth hormones, proven to be an excellent plant growth enhancer. Primed seeds helps for rapid and uniform germination might result in vigorous seedlings. The results are in conformity with the findings of [12] in sorghum, [13] in ragi, and [23] in rice.

CONCLUSION

The study clearly revealed that barnyard millet seeds primed with moringa leaf extract @ 5% for 6 h and stored in Aluminum container maintained its germination for minimum seed certification standard till the end of the storage period in barnyard millet cv. CO 1.

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Table - 1. Effect of various seed priming treatments, storage containers and period of storage on germination (%) in Barnyard millet cv. CO 1

Containers	Treatments	P_0	P_2	P_4	P_6	P_8	P_{10}	P_{12}	MEAN
C_1	T_0	88	85	82	79	78	74	70	79
		(69.74)	(67.22)	(64.90)	(62.74)	(62.03)	(59.35)	(56.80)	(63.26)
	T_1	90	87	80	79	75	73	72	79
		(71.58)	(68.88)	(63.44)	(62.73)	(60.00)	(58.70)	(58.06)	(63.34)
	T_2	89	87	83	80	78	77	71	81
		(70.64)	(68.91)	(65.68)	(63.45)	(62.04)	(61.36)	(57.42)	(64.21)
	T_3	91	90	86	84	79	76	73	83
		(72.56)	(71.58)	(68.04)	(66.43)	(62.73)	(60.68)	(58.71)	(65.82)
	T_4	96	92	89	82	80	79	77	85
		(78.72)	(73.65)	(70.64)	(64.90)	(63.44)	(62.73)	(61.35)	(67.92)
	T_5	91	88	85	82	77	74	72	81
		(72.56)	(69.74)	(67.22)	(64.90)	(61.35)	(59.35)	(58.06)	(64.74)
	T_6	92	90	87	83	80	76	74	83
		(73.65)	(71.58)	(68.88)	(65.66)	(63.44)	(60.67)	(59.35)	(66.18)
	T_7	90	87	85	84	79	77	73	82
		(71.58)	(68.88)	(67.22)	(66.43)	(62.73)	(61.35)	(58.70)	(65.27)
	T_8	89	85	83	80	78	72	71	80
		(70.64)	(67.22)	(65.66)	(63.44)	(62.04)	(58.06)	(57.42)	(63.50)
	MEAN	91	88	84	81	78	75	73	82
		(72.41)	(69.74)	(66.85)	(64.52)	(62.20)	(60.25)	(58.43)	(64.92)
	T_0	90	88	84	83	80	79	78	83
		(71.62)	(69.78)	(66.45)	(65.66)	(63.44)	(62.74)	(62.04)	(65.96)
	T_1	91	90	87	85	84	83	82	86
		(72.61)	(71.62)	(68.88)	(67.22)	(66.43)	(65.66)	(64.90)	(68.19)
	T_2	93	90	88	83	82	80	79	85
		(74.69)	(71.58)	(69.74)	(65.66)	(64.90)	(63.44)	(62.73)	(67.54)
	T_3	92	91	90	89	87	86	84	88
		(73.65)	(72.56)	(71.58)	(70.64)	(68.88)	(68.04)	(66.43)	(70.26)
	T_4	97	95	94	92	91	89	88	92
		(80.12)	(77.12)	(75.86)	(73.65)	(72.56)	(70.64)	(69.74)	(74.24)
	T_5	93	92	90	89	88	85	83	85
		(74.69)	(73.59)	(71.58)	(70.64)	(69.74)	(67.22)	(65.66)	(67.22)

Containers	Treatments	P_0	P_2	P_4	P_6	P_8	P_{10}	P_{12}	MEAN
C_2	T_6	94	92	91	89	88	87	85	89
		(75.95)	(73.65)	(72.61)	(70.64)	(69.74)	(68.88)	(67.22)	(71.24)
	T_7	92	90	88	87	85	82	81	86
		(73.59)	(71.58)	(69.74)	(68.88)	(67.22)	(64.92)	(64.16)	(68.59)
	T_8	91	89	87	86	83	82	80	85
		(72.56)	(70.64)	(68.91)	(68.06)	(65.68)	(64.90)	(63.44)	(67.74)
	MEAN	93	91	89	87	85	84	82	87

		(74.39)	(72.46)	(70.59)	(69.01)	(67.62)	(66.27)	(65.15)	(69.36)
Treatment mean	T_0	89	87	83	81	79	77	74	81
		(70.68)	(68.50)	(65.68)	(64.20)	(62.74)	(61.05)	(59.42)	(64.61)
	T_1	91	89	84	82	80	78	77	83
		(72.10)	(70.25)	(66.16)	(64.98)	(63.22)	(62.18)	(61.48)	(65.77)
	T_2	91	89	86	82	80	79	75	83
		(72.67)	(70.24)	(67.71)	(64.56)	(63.47)	(62.40)	(60.08)	(65.87)
	T_3	92	91	88	87	83	81	79	86
		(73.11)	(72.07)	(69.81)	(68.50)	(65.80)	(64.36)	(62.57)	(68.04)
	T_4	97	94	92	87	86	84	83	89
		(79.42)	(75.39)	(73.25)	(68.50)	(68.00)	(66.69)	(65.55)	(71.08)
	T_5	92	90	88	86	83	80	78	85
		(73.62)	(71.67)	(69.40)	(67.77)	(65.55)	(63.29)	(61.86)	(67.59)
	T_6	93	91	89	86	84	82	80	87
		(74.80)	(72.62)	(70.74)	(68.15)	(66.59)	(64.77)	(63.29)	(68.50)
	T_7	91	89	87	86	82	80	77	84
		(72.59)	(70.23)	(68.48)	(67.65)	(64.98)	(63.13)	(61.43)	(66.93)
	T_8	90	87	85	83	81	77	76	83
		(71.60)	(68.93)	(67.28)	(65.75)	(63.86)	(61.48)	(60.43)	(65.62)
	MEAN	92	89	87	84	82	80	77	84
		(73.40)	(71.10)	(68.72)	(66.76)	(64.91)	(63.26)	(61.79)	(67.14)
Level of significance									
		C	**T**	**P**	**C x T**	**T x P**	**C x P**	**C x P x T**	
SEd		0.136 (0.118)	0.288 (0.250)	0.254 (0.220)	0.407 (0.353)	0.761 (0.661)	0.359 (0.312)	1.076 (0.935)	
CD (P = 0.05)		0.267 (0.232)	0.567 (0.492)	0.500 (0.434)	0.801 (0.696)	1.499 (1.302)	0.707 (0.614)	2.120 (1.842)	

Figures in parenthesis are Arcsine Transformed value

Table - 2. Effect of various seed priming treatments, storage containers and period of storage on root length (cm) in Barnyard millet cv. CO 1

Containers	**Treatments**	P_0	P_2	P_4	P_6	P_8	P_{10}	P_{12}	MEAN
C_1	T_0	11.51	11.08	10.69	10.16	9.75	9.58	9.32	10.30
	T_1	12.67	12.17	11.76	11.22	10.74	10.46	10.19	11.32
	T_2	11.89	11.23	10.81	10.47	10.12	9.95	9.71	10.60
	T_3	12.33	11.85	11.61	11.32	11.18	11.04	10.79	11.45
	T_4	13.95	13.32	12.79	12.45	12.03	11.87	11.58	12.57
	T_5	13.28	12.84	12.15	11.56	10.57	10.19	9.94	11.50
	T_6	13.71	13.27	12.75	12.24	11.84	11.5	11.22	12.36
	T_7	12.96	12.16	11.72	11.38	10.92	10.68	10.45	11.47
	T_8	12.14	11.66	11.23	10.84	10.39	9.82	9.56	10.81
	MEAN	12.72	12.18	11.72	11.29	10.84	10.57	10.31	11.37

C_2	T_0	11.75	11.25	10.79	10.33	10.09	9.80	9.61	10.52
	T_1	12.09	11.89	11.54	11.21	10.76	10.49	10.34	11.19
	T_2	13.67	13.28	12.75	11.49	10.65	10.06	9.86	11.68
	T_3	13.2	13.04	12.93	12.72	12.57	12.31	12.13	12.70
	T_4	14.56	14.22	13.71	13.34	13.16	12.92	12.78	13.53
	T_5	12.78	12.53	12.3	11.63	11.23	10.81	10.67	11.71
	T_6	14.11	13.91	13.65	13.29	12.98	12.73	12.51	13.31
	T_7	13.89	13.58	12.78	12.56	11.72	11.47	11.25	12.46
	T_8	12.41	12.26	12.21	12.11	12.07	11.95	11.84	12.12
	MEAN	13.16	12.88	12.52	12.08	11.69	11.39	11.22	12.14
Treatment mean	T_0	11.63	11.17	10.74	10.25	9.92	9.69	9.47	10.41
	T_1	12.38	12.03	11.65	11.22	10.75	10.48	10.27	11.25
	T_2	12.78	12.26	11.78	10.98	10.39	10.01	9.79	11.14
	T_3	12.77	12.45	12.27	12.02	11.88	11.68	11.46	12.07
	T_4	14.26	13.77	13.25	12.90	12.60	12.40	12.18	13.05
	T_5	13.03	12.69	12.23	11.60	10.90	10.50	10.31	11.61
	T_6	13.91	13.59	13.20	12.77	12.41	12.12	11.87	12.84
	T_7	13.43	12.87	12.25	11.97	11.32	11.08	10.85	11.97
	T_8	12.28	11.96	11.72	11.48	11.23	10.89	10.70	11.46
	MEAN	12.94	12.53	12.12	11.68	11.27	10.98	10.76	11.75
Level of significance									
		C	T	P	C x T	T x P	C x P	C x P x T	
SEd		0.057	0.123	0.108	0.173	0.324	0.153	0.459	
CD (P = 0.05)		0.114	0.241	0.213	0.341	0.639	0.301	0.903	

Table - 3. Effect of various seed priming treatments, storage containers and period of storage on shoot length (cm) in Barnyard millet cv. CO 1

Containers	**Treatments**	P_0	P_2	P_4	P_6	P_8	P_{10}	P_{12}	MEAN
C_1	T_0	7.61	7.49	7.15	6.97	6.68	6.32	6.11	6.90
	T_1	7.95	7.86	7.46	7.08	6.79	6.55	6.30	7.14
	T_2	8.17	8.09	7.81	7.49	7.12	6.87	6.63	7.45
	T_3	7.74	7.62	7.54	7.41	7.20	7.05	6.92	7.35
	T_4	8.76	8.69	8.44	8.21	7.83	7.34	7.18	8.06
	T_5	8.38	8.30	8.13	7.89	7.45	6.92	6.74	7.69
	T_6	8.51	8.42	8.24	7.72	7.41	7.16	7.01	7.78
	T_7	8.29	8.11	7.87	7.56	7.22	6.98	6.87	7.56
	T_8	8.10	7.97	7.76	7.53	7.18	6.82	6.55	7.42
	MEAN	8.17	8.06	7.82	7.54	7.21	6.89	6.70	7.48
C_2	T_0	7.76	7.71	7.52	7.31	7.11	6.77	6.41	7.23
	T_1	8.13	8.08	7.93	7.82	7.68	7.34	7.11	7.73
	T_2	8.49	8.42	8.15	7.69	7.28	6.86	6.63	7.65
	T_3	7.85	7.78	7.64	7.51	7.36	7.15	6.92	7.46

	T_4	8.92	8.87	8.72	8.54	8.29	8.10	7.92	8.48
	T_5	8.63	8.54	8.33	8.11	7.89	7.67	7.45	8.09
	T_6	8.71	8.66	8.49	8.32	8.14	7.93	7.70	8.28
	T_7	7.97	7.92	7.78	7.63	7.48	7.33	7.21	7.62
	T_8	8.28	8.19	7.84	7.61	7.35	7.13	6.89	7.61
	MEAN	8.30	8.24	8.04	7.84	7.62	7.36	7.14	7.79
Treatment mean	T_0	7.69	7.60	7.34	7.14	6.90	6.55	6.26	7.07
	T_1	8.04	7.97	7.70	7.45	7.24	6.95	6.71	7.43
	T_2	8.33	8.26	7.98	7.59	7.20	6.87	6.63	7.55
	T_3	7.80	7.70	7.59	7.46	7.28	7.10	6.92	7.41
	T_4	8.84	8.78	8.58	8.38	8.06	7.72	7.55	8.27
	T_5	8.51	8.42	8.23	8.00	7.67	7.30	7.10	7.89
	T_6	8.61	8.54	8.37	8.02	7.78	7.55	7.36	8.03
	T_7	8.13	8.02	7.83	7.60	7.35	7.16	7.04	7.59
	T_8	8.19	8.08	7.80	7.57	7.27	6.98	6.72	7.51
	MEAN	8.24	8.15	7.93	7.69	7.41	7.13	6.92	7.64
Level of significance									
		C	T	P	C x T	T x P	C x P	C x P x T	
SEd		0.013	0.028	0.025	0.040	0.075	0.035	0.105	
CD (P = 0.05)		0.026	0.055	0.049	0.078	0.147	0.069	0.208	

Table - 4. Effect of various seed priming treatments, storage containers and period of storage on Dry matter production (g. seedlings^{-10}) in Barnyard millet cv. CO 1

Containers	**Treatments**	P_0	P_2	P_4	P_6	P_8	P_{10}	P_{12}	MEAN
C_1	T_0	0.019	0.018	0.016	0.015	0.014	0.012	0.011	0.015
	T_1	0.021	0.020	0.018	0.017	0.015	0.013	0.013	0.017
	T_2	0.020	0.018	0.017	0.016	0.016	0.014	0.012	0.016
	T_3	0.023	0.022	0.021	0.020	0.019	0.018	0.018	0.020
	T_4	0.027	0.026	0.025	0.023	0.023	0.022	0.021	0.024
	T_5	0.022	0.020	0.019	0.019	0.018	0.018	0.017	0.019
	T_6	0.025	0.024	0.024	0.022	0.021	0.020	0.019	0.022
	T_7	0.024	0.022	0.021	0.018	0.018	0.016	0.015	0.019
	T_8	0.023	0.022	0.021	0.020	0.017	0.015	0.014	0.019
	MEAN	0.023	0.021	0.020	0.019	0.018	0.016	0.016	0.022
C_2	T_0	0.021	0.020	0.019	0.018	0.018	0.016	0.015	0.018
	T_1	0.022	0.021	0.021	0.020	0.020	0.019	0.018	0.020
	T_2	0.025	0.024	0.022	0.021	0.019	0.018	0.017	0.021
	T_3	0.024	0.023	0.023	0.022	0.022	0.021	0.021	0.022
	T_4	0.030	0.029	0.027	0.027	0.026	0.025	0.025	0.027
	T_5	0.026	0.024	0.024	0.022	0.020	0.019	0.018	0.022
	T_6	0.027	0.026	0.025	0.025	0.023	0.023	0.022	0.024
	T_7	0.024	0.022	0.022	0.021	0.021	0.020	0.020	0.021

	T_8	0.023	0.021	0.020	0.019	0.019	0.017	0.016	0.019
	MEAN	0.025	0.023	0.023	0.022	0.021	0.020	0.019	0.024
Treatment mean	T_0	0.020	0.019	0.018	0.017	0.016	0.014	0.013	0.017
	T_1	0.022	0.021	0.020	0.019	0.018	0.016	0.016	0.018
	T_2	0.023	0.021	0.020	0.019	0.018	0.016	0.015	0.019
	T_3	0.024	0.023	0.022	0.021	0.021	0.020	0.020	0.021
	T_4	0.029	0.028	0.026	0.025	0.025	0.024	0.023	0.025
	T_5	0.024	0.112	0.022	0.021	0.019	0.019	0.018	0.020
	T_6	0.026	0.025	0.025	0.024	0.022	0.022	0.021	0.023
	T_7	0.024	0.022	0.022	0.020	0.020	0.018	0.018	0.020
	T_8	0.023	0.022	0.021	0.020	0.018	0.016	0.015	0.019
	MEAN	0.024	0.032	0.021	0.020	0.019	0.018	0.017	0.022
Level of significance									
		C	T	P	C x T	T x P	C x P	C x P x T	
SEd		0.0002	0.0003	0.0003	0.0005	0.0009	0.0004	0.0012	
CD (P = 0.05)		0.0003	0.0006	0.0006	0.0009	0.0017	0.0008	0.0024	

Table - 5. Effect of various seed priming treatments, storage containers and period of storage on vigour index in Barnyard millet cv. CO 1

Containers	**Treatments**	P_0	P_2	P_4	P_6	P_8	P_{10}	P_{12}	MEAN
C_1	T_0	1685	1581	1465	1355	1283	1178	1082	1376
	T_1	1857	1744	1539	1447	1316	1243	1188	1476
	T_2	1788	1683	1548	1439	1347	1297	1162	1466
	T_3	1830	1756	1650	1576	1455	1377	1295	1563
	T_4	2182	2027	1891	1696	1590	1519	1446	1764
	T_5	1976	1865	1728	1599	1391	1269	1204	1576
	T_6	2047	1955	1829	1659	1542	1420	1351	1686
	T_7	1915	1766	1667	1593	1435	1362	1266	1572
	T_8	1805	1672	1579	1472	1373	1200	1146	1464
	MEAN	1898	1783	1655	1537	1415	1318	1238	1549
C_2	T_0	1758	1671	1540	1466	1378	1311	1251	1482
	T_1	1842	1799	1695	1619	1550	1481	1432	1631
	T_2	2064	1956	1842	1594	1472	1355	1305	1655
	T_3	1940	1898	1855	1804	1737	1677	1603	1788
	T_4	2280	2195	2110	2015	1954	1872	1823	2036
	T_5	1996	1943	1862	1761	1687	1575	1508	1762
	T_6	2148	2079	2018	1926	1861	1800	1720	1936
	T_7	2014	1938	1812	1759	1634	1544	1497	1743
	T_8	1886	1823	1748	1699	1615	1567	1501	1691
	MEAN	1992	1923	1831	1738	1654	1576	1516	1747
me nt me	T_0	1722	1626	1503	1411	1331	1245	1166	1429
	T_1	1850	1771	1617	1533	1433	1362	1310	1554

	T_2	1926	1819	1695	1516	1409	1326	1233	1561
	T_3	1885	1827	1752	1690	1596	1527	1449	1675
	T_4	2231	2111	2001	1855	1772	1696	1634	1900
	T_5	1986	1904	1795	1680	1539	1422	1356	1669
	T_6	2098	2017	1923	1792	1702	1610	1536	1811
	T_7	1965	1852	1740	1676	1535	1453	1382	1657
	T_8	1846	1748	1663	1586	1494	1384	1324	1578
	MEAN	1945	1853	1743	1638	1534	1447	1377	1648
Level of significance									
		C	T	P	C x T	T x P	C x P	C x P x T	
SEd		16.132	34.222	30.181	48.397	90.542	42.682	128.046	
CD (P = 0.05)		31.772	67.399	59.441	95.317	178.322	84.062	252.185	

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RESEARCH ARTICLE

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) Determination of Lemborexant in Biological Specimens

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ABSTRACT

Lemborexantis a new chemical entity used in the treatment of epilepsy treatment. In the present investigation, a rapid, specific, selective and novel method has been optimized for evaluation of Lemborexantin plasma using Trazodoneas an internal standard by LC-MS/MS. The Lemborexant and Trazodone extracted from human plasma using liquid-liquid extraction method using tertiary-Butyl methyl ether as extraction solvent. The principle analytes were eluted with the conditions of mobile phase having the 0.1% Formic acid : Acetonitrile (20:80%, v/v) using the Eclipse Plus C_{18}, 4.6 mm × 150 mm, (5 µm) analytical column with the 0.6 ml/min flow rate and 10 µl sample volume using CEM array detector. The retention times of Lemborexant and Trazodone were 1.65±0.05 min 0.58±0.05 min with the total run time of 4 min. The curve indicates correlation coefficient (r^2) was superior by having the value nearer to 1.0 with linear range of 10.0 ng/ml to 500.0ng/ml. The correlation coefficient (r^2) for Lemborexant was found to be 0.999. The LOQ and LOD for the Lemborexant was found to be 1.03 ng/ml and 0.34 ng/ml. The method was validated to comply with the United States Food and Drug Administration (USFDA) rules by evaluating system suitability, specificity, sensitivity, linearity, precision, accuracy, roughness and stability. The verification parameter results were found to be within acceptable limits. Thus the method which has been originally developed can be used for routine Lemborexant measurement in plasma samples.

Keywords: Lemborexant, Trazodone, Plasma, Validation, USFDA, Bio-analytical, LC-MS/MS.

INTRODUCTION

Lemborexant, an orexin receptor antagonist. The chemical name of lemborexant is (1R,2S)-2-{[(2,4-dimethylpyrimidin-5-yl)oxy]methyl}-2-(3-fluorophenyl)-*N*-(5-fluoropyridin-2-yl) cyclopropanecarboxamide. The molecular formula is $C_{22}H_{20}F_2N_4O_2$. The molecular weight is 410.42. [1-9]: Lemborexant is a white to off-white powder that is practically insoluble in water. Lemborexant is a novel dual orexin receptor antagonist recently approved by the US Food and Drug Administration to treat insomnia and in development to treat irregular sleep/wake rhythm disorder. In-vitro and in-vivo studies demonstrated preclinical pharmacological activities of lemborexant with potent and selective binding affinities to orexin receptors [2,3] and a double-blind placebo controlled clinical study also suggested that lemborexant was effective and safe in humans [4,5]. The present study focuses to develop bioanalytical methods for the determination of Lemborexant in human plasma. For the estimation of the drugs present in the biological fluid, LC–MS/MS method is considered to be more suitable since this is a powerful and rugged method [10]. It is also extremely specific, linear, precise, accurate, sensitive, and rapid. The developed method could then be applied to clinical trials to obtain accurate pharmacokinetic parameters in human plasma [11].

MATERIALS AND METHODS

Lemborexant and Trazodone (Figure1), acetonitrile (J.T.Baker, Phillipsburg, NJ, USA), water (Milli - Q system, Millipore, Bedford, MA, USA), Formic acid (Merck Pvt. Ltd, Worli, Mumbai).

Standards and quality control samples for spiked plasma

Standard stock solutions for Lemborexant and Trazodone were prepared by taking 10 mg in a 10ml volumetric flask, 10ml of acetonitrile was added, sonicating for 3 minutes and then final volume is made up to 10 ml with the diluent. To perform validation parameters, all standard solutions were spiked with 50ng/ml Trazodone. Lemborexant (10 ng/ml to 500 ng/ml) and Trazodone (50 ng/ml) were prepared from standard stock solutions. Calibration curve standards were prepared to obtain 10.0, 20.0, 40.0, 60, 80.0, 100, 200, 400 and 500 ng/ml of Lemborexant and 50 ng/ml of Trazodone in drug-free plasma (0.2m.l). Four quality control samples with concentrations of 10 ng/ml (LLOQ), 30 ng/ml (LQC), 250 ng/ml (MQC) and 400 ng/ml (HQC) were prepared by spiking Lemborexant-free plasma. In all quality control samples, Trazodone is spiked at 50 ng/ml. Calibration and quality control samples were stored at -80^{o}.

Processing of plasma samples

The extraction technique for Lemborexant and Trazodone from plasma samples was applied. At room temperature, the linearity and quality control samples are thawed and homogenized with a vortex mixer 2 ml of diethyl ether and 10µlof 0.1 % Formic acid are added to 200µl of spiked plasma samples. The sample was mixed in a vortex mixer for 10 seconds. The blend was vortexed for 2 minutes. The mixture was finally centrifuged for 10 min at 4500 rpm. After centrifugation, the organic layer was dried and reconstructed in the mobile phase. The samples were transferred into the LC-MS / MS system in auto sampler vials for injection (10 µl).

Optimization of Chromatographic conditions and mass spectrometric

After series of trials, the chromatographic conditions were accomplished with the 0.1% Formic Acid: Acetonitrile (20:80%, v/v) by utilizing the stationary phase Eclipse Plus C_{18}, 4.6 mm × 150 mm, (5 µm) to obtain the best peak shape with the column temperature 40°C and sample compartment temperature of 10° C with the flow rate of 0.6 ml/min with the sample volume of 10µl. Lemborexant and Trazodone (Internal standard) analysis was performed using MRM positive ion mode with mass transitions of m/z (amu) 411.2→287.2 and 372.1→176.2. The mass spectrum of parent and product ions were depicted in Fig.2.

VALIDATION OF ANALYTICAL METHOD

Validation was performed as per USFDA guidelines for the developed method with the stringent limit to prove the efficiency of this method [12-14].

System precision

In keeping with the internal protocol, mid-quality control (MQC) samples were inserted six times into the HPLC device to test the suitability of the device. Percentage of the relative standard deviation for Lemborexant and Trazodone (internal standard) peak area response and retention period was measured. The percentage of the relative variance for the peak area ratio was also calculated.

Method specificity

Specificity has been used to demonstrate that chromatographically, blank plasma components should not intervene. Six blank plasma samples and LLOQ (10ng/ml) were analysed, Interference with analyte samples was tested. The maximum response of each interruption peak should not bemore than 20% Lemborexant and 5% internal normal peak area. The sensitivity test at the LLOQ concentration range (10 ng/ml) was conducted to detect the lowest detection level. To this end, LLOQ samples have been inserted six times into the LC-MS device and the percentage of proportional standard deviation has been determined.

Method linearity

10µl of calibration curve solutions (10-500 ng/ml) have been pumped into the column three times. The chromatograms were reported with optimized chromatographic conditions. The concentration of the unknown was determined using regression equation derived by the peak response ratio and the concentration data.

Method precision

Accuracy and precision were established in six replicates in six runs, by analysing samples of quality control at four concentration levels (LLOQ, LQC, MQC and HQC). The recovery percentage was used to evaluate accuracy and to evaluate precision with a relative standard deviation. The acceptable inter-day and intra-day precision criterion is 15% for LQC, MQC and HQC and 20% for LLOQ samples. The acceptable method accuracy criterion was ±15% for LQC, MQC and HQC and ±20% for LLOQ samples. Table 1 illustrates the inter- and intra-day accuracy results of four quality control samples.

Robustness

To verify the method efficiency when the minor changes happened in the optimized method parameters like mobile phase flow rate and column temperature parameters was performed with the criteria, the method should pass the system suitability criteria.

The detection limit (LOD) and quantification limit (LOQ)

By comparing test results from samples with known concentrations of analytes with blank samples, LODs and LOQ were determined using a signal-to-noise ratio (S / N).

Recovery analyte

Lemborexant and Trazodone were analyzed by spiking three levels (LQC, MQC and HQC) into blank plasma samples. Recovery of the analyte was determined by comparing the peak area obtained by un-extracted samples from Lemborexant and Trazodone (internal standard). The criterion of acceptance was that the relative standard deviation in recovery at every level of quality control and internal standard should be less than 15 percent.

Matrix effect

Matrix effects were quantified by determining the matrix factor to forecast the variability of matrix effects in samples from individual subjects. Each triplicate was extracted with six lots of blank biological matrices and post spiked with

aqueous quality control (MQC) level, and compared with the same concentration aqueous standards. The overall matrix factor precision was expressed as a variation coefficient (CV %), and CV% is < 15%.

Assessment of stability of standard stock solutions

The prepared stock standards of Lemborexant and Trazodone (Internal standard) were verified up to 48 hours for the stability at ambient and refrigerated conditions.

Sample stability

In different study conditions, the stability of Lemborexant in plasma was evaluated at room temperature over 24 hrs and store for one month at −80°C (long-term stability). The results of Lemborexant stability in plasma under different storage conditions were expressed as a percentage recovery and relative standard deviation. By comparing the stability sample with newly spiked samples, the percentage stability of Lemborexant stored at -80°C was assessed. The stability studies were carried out using samples of LQC (30 ng/ml) and HQC (400 ng/ml). The exact percentage should be within 85-115 percent and the relative standard deviation of the percentage should be less than 5 percent.

RESULTS

From figure 3 there was a clear separation and good resolution and without any carryover was achieved with this method and the system suitability acceptance criteria also found satisfactory. For the system precision parameter the %RSD was achieved as 0.04% against the limit NMT 2.0%. The peak area response in all the six blank plasma samples is zero. The results demonstrated the non-interference from blank plasma components (Fig. 3). Hence the method is selective and specific. The % RSD of Lemborexant at LLOQ level was 1.48%. The value was found to be within the acceptance limit (%RSD - ≤20%). Therefore, the proposed was sensitive. The data in table-1 indicates acceptable accuracy and precision (%RSD was NMT 2.0%) for both intra-day and inter-day samples at all the four concentration levels. The linearity parameter was quantified by peak area ratio against concentration methodology. Different concentrations from 10 ng/ml to 500 ng/ml standard solutions were prepared and injected into the system. The calculated regression coefficient was 0.999 as shown in the Figure 4. To evaluate the method capability of producing precise results with the minor variations of flow and column temperature variations as robustness was performed. The results were shown in the Table 2. The results proving that the method was stable to produce consistent results with the minor variation of the method parameters. The LOQ and LOD were identified by injecting the lower concentrations with the S/N ratio criteria. The LOD and LOQ for the Lemborexant was 0.34 ng/ml and 1.03 ng/ml. The recovery results are summarized in Table 3. The values are within the acceptance limits. The method provided good extraction efficiency for Lemborexant and internal standard in plasma samples. The overall precision of the matrix factor is 0.42 for Lemborexant. There was no ion-suppression and ion-enhancement effect observed due to IS and anal teat respective retention times(Fig-3).As shown in Table 4 and 5, the stability results are within the acceptance limits. The results indicated that Lemborexant was stable for the complete period of analysis.

DISCUSSION

During method optimization initially organic solvents used as mobile phase with the water in different composition. But both compounds were not detected. Then started usage of buffer with organic solvent such as acetonitrile in different ratios and pH with the Eclipse PlusC_{18}, 4.6 mm × 150 mm, (5 µm) column. Finally the method was found optimized with the conditions of mobile phase (0.1% Formic Acid: Acetonitrile (20:80% v/v) by utilizing the stationary phase flow rate of 0.6 ml/min, column temperature of 40°C, sample compartment temperature of 10°C, sample volume of 10 µl. With this method both actives Lemborexant and Trazodone eluted at 1.65±0.05 min 0.58±0.05 min with good resolution and symmetry. After the method optimization the method was validated as per USFDA guidelines. As per the results obtained in the method validation there was no interference of the blank and carryover problem even at the LLOQ level quantification. Both LOQ and LOD of this method were verified

practically in the instrument with S/N ratio criteria. The results shows method was high sensitive. Hence, this method can be applicable during product optimization at the time of pharmacokinetic studies.

CONCLUSION

The LC-MS / MS method was developed and validated for the quantification of Lemborexantin human plasma according to regulatory instructions, with highly sensitive, specific, reproductive, rapid and high output ratings. The current approach implied a simple sample extraction method that provided consistent and reproducible retrievals. Results obtained show that the strategy proposed can be easily and applied favorably to the Lemborexantroutine in the plasma of human beings. It can be applied due to the sensitivity of the method developed plasma level monitoring in preclinical drug analysis and pharmacokinetic clinical trials. All results and validation parameters have been found within the acceptance limit.

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CONFLICT OF INTEREST: Authors assuring that, there is no conflict of interest.

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Table 1.Ruggedness Data of Lemborexant

QC levels*	LLOQ (10 ng/ml)	LQC (30 ng/ml)	MQC (50 ng/ml)	HQC (80 ng/ml)
Intra-day precision and accuracy				
Mean calculated (ng/ml)**	9.78	29.67	247.06	398.66
RSD (%)	4.10	5.72	0.84	0.22
Accuracy (%)	97.76	98.89	98.82	99.67
Inter-day precision and accuracy				
Mean calculated (ng/ml)**	9.74	28.51	246.68	395.51
RSD (%)	5.82	6.40	1.92	2.85
Accuracy (%)	97.37	95.03	98.67	98.88

* QCS – Quality control samples

** Mean of six determinations

Table. 2. : Robustness data of Lemborexant

Parameter	Flow Rate (+5%)	Flow Rate (-5%)	Column Temp High (+2°C)	Column Temp Low (-2°C)
Mean**	251.1	250.86	251.154	251.95
SD	0.76	1.06	0.54	1.20
% RSD	0.30	0.42	0.21	0.48
Mean %RSD	0.36		0.35	

** Mean of six determinations

Table 3: Recovery data

Parameter	Lemborexant	Trazodone
(LQC)		
Mean Peak Area*	16360	330957
SD(±)	16.84	190.82
% RSD	0.10	0.06
% Recovery	89.20	93.59
MQC		
Mean Peak Area*	924621	330753
SD(±)	54.25	190.82

% RSD	0.01	0.06
% Recovery	90.30	93.00
HQC		
Mean Peak Area*	1878320	328780
SD(±)	263.79	4209.88
% RSD	0.01	1.28
% Recovery	94.94	91.66
Mean %recovery	91.48	92.75
Mean %CV	0.05	0.71

*Average of six determinations

Table.4.0: Stability data of standard solutions

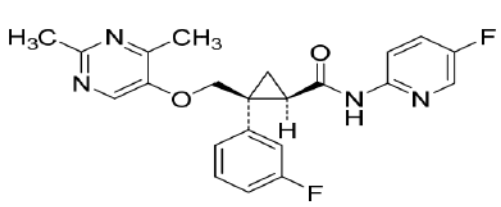
Parameter	**Lemborexant**		**Trazodone**	
	***Comparison samples**	***ST Stability samples**	***Comparison samples**	***ST Stability samples**
Mean Peak Area*	910419.67	854746.33	397709.67	327381.67
SD (±)	10528.43	37847.16	3385.60	12069.39
CV (%)	1.16	4.43	0.85	3.69
Mean %Accuracy	98.51		95.54	

* Mean of six determinations

Table 5: Summary of stability of Lemborexant in plasma under different storage conditions

Quality control sample	**Concentration of Lemborexant (ng/ml)**	
	LQC*	**HQC***
Freeze and Thaw stability (-80°C)		
%CV	13.68	2.07
%Accuracy	91.41	98.17
Room temperature stability (24 hours)		
%CV	1.62	2.15
%Accuracy	95.18	94.95
Auto Sampler Stability (24 hours)		
%CV	2.47	1.83
%Accuracy	94.77	97.00
Re-Injection Stability (40 Hours)		
%CV	8.09	1.61
%Accuracy	94.32	98.22
Long term stability (1 month; -80 °C)		
%CV	1.79	2.80
%Accuracy	94.24	97.75

*Average of six determinations



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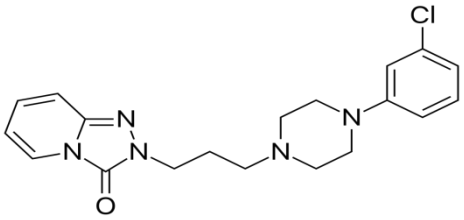
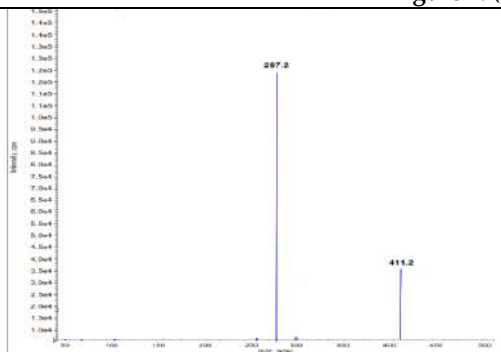
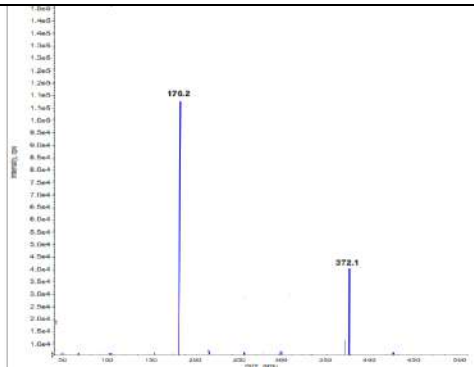


(b)

Figure 1. (A) Lemborexant (B) Trazodone



(A)



(B)

Figure. 2. Mass chromatograms of (A) Lemborexant and (B) Trazodone

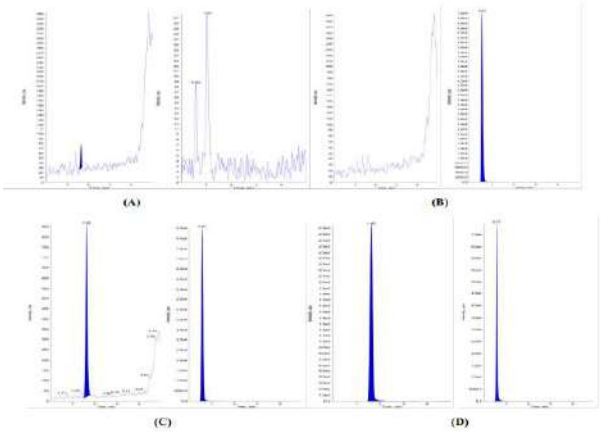


Figure. 3. MRM chromatograms of (A) Blank human plasma (B) Blank plasma spiked with Trazodone (50 ng/ml) as internal standard (IS) (C) Plasma spiked with LLOQ Standard (10 ng/ml) and Trazodone (IS) 50 ng/ml) (D) Plasma spiked with U.L.O.Q standard (500 ng/ml) and Trazodone (IS) 50 ng/ml). Sensitiveness

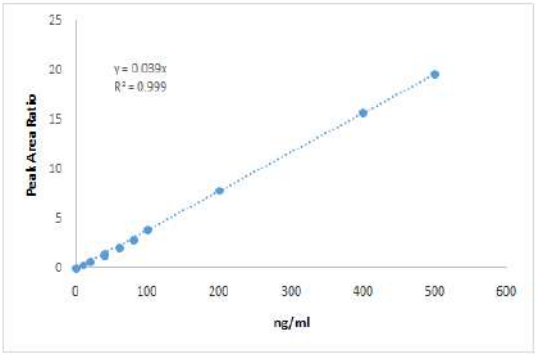


Figure.4: Linearity data

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RESEARCH ARTICLE

Effect of Muriate of Potash on Yield and Economics of Blackgram (*Vigna mungo* L*)*

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ABSTRACT

The field experiment was conducted at farmer's field in Sivapuri village, Chidambaram taluk, Cuddalore district, Tamilnadu. The experimental soil was clay loam with a pH of 7.90, EC of 0.86 dSm^{-1} and CEC of 22.4 c mol (p^+) kg^{-1}. The available N, P, K content of soil were 188, 10.0, 117 kg ha^{-1} respectively. Thus the fertility status of K comes under low status. The experimental design adopted in the study was randomized block design with three replications and eight treatments. The treatments were T_1 - Absolute control, T_2 - Control (-K), T_3 - 12.5 kg of K_2O ha^{-1}, T_4 – 25 kg of K_2O ha^{-1}, T_5 – 37.5kg of K_2O ha^{-1}, T_6 - 50kg of K_2O ha^{-1}, T_7 – 62.5kg of K_2O ha^{-1}, T_8 - 75kg of K_2O ha^{-1}. The growth and yield parameters were observed and recorded. The results of the experiment indicated that application of T_6 –50 kg ha^{-1} of K_2O significantly enhanced the yield and economics of blackgram, further were reduced due to excess application of potassium. The treatment T_6 recorded grain and haulm yield of 1080 kg ha^{-1}, 1483 kg ha^{-1}respectively and benefit cost ratio of 3.18. However the treatment T_6 and T_7 were statistically on par. The other treatments which were found to be statistically significant.

Keywords: Economics of blackgram, N, P, K content of soil, yield, treatments, parameters.

INTRODUCTION

Blackgram [*Vigna mungo* (L.) Hepper] is the third important pulse crop of India which is cultivated over a wide range of agro-climatic zones of the country. It grows well in both abnormal and normal weather situation. It occupies about 3.25 million ha area in the country producing 1.5 million tones of seed with average productivity of 462 kg/ha (AICRP, 2013). Kota district of Rajasthan occupies 13441 ha area with average productivity of 800 kg/ha of urd which is slightly higher against the Rajasthan average productivity of 516 kg/ha (GOR, 2012) .In Tamil Nadu, it is cultivated in 4.56 lakh hectares of area with a production of 2.36 lakh tones and the average productivity is 518 kg ha^{-1}. The average productivity of pulses in Tamilnadu is very low when compared to India's average of 610 kg ha^{-1}.

Production of black gram is low in general due to poor management and low soil fertility status. Madhya Pradesh, Maharashtra, Uttar Pradesh, Tamil Nadu, Orissa and Gujarat are the main black gram growing states of India. The productivity potential of pulses are not realized and the reasons for low productivity of blackgram are large scale cultivation under rainfed and marginal lands and may be under low input conditions (Rathore, 2002). The productivity of pulse crops including blackgram is not sufficient enough to meet the domestic demand of the population. Hence, there is need for enhancement of the productivity of blackgram by proper practices. K plays an important role in the maintenance of electrical potential gradients across cell membranes and the generation of turgor. It is also essential for photosynthesis, protein synthesis and regulation of stomatal movement and it is the major cation in maintenance of cation-anion balances (Marschner, 1995). Blackgram plays an important role in sustaining soil fertility by improving soil physical properties and fixing atmospheric nitrogen. It is a drought resistant crop and suitable for dry land farming and predominantly used as an intercrop with other crops. It is mostly consumed in southern India. Considering its nutritional value and price, it is necessary to raise its production level and nutritional quality. In contrastless work has been undertaken with the consideration of other plant nutrients, thus, now days it is necessary to emphasize potassium study in pulses and blackgram. However, the poor nutritional management of black gram is one of the major constraints for exploring the yield potential of this crop.

MATERIALS AND METHODS

Field experiment was conducted in the farmer's field at Sivapuri village near Chidambaram, Cuddalore district, Tamil Nadu. The experimental farm is geographically situated at 11°38' North latitude and 79°70' East longitude and at an altitude of ±5.79 m above mean sea level and 6 km away from Bay of Bengal. The soils of Sivapuri village was found to contain soil separates of 29.2, 39.4, 30.5 percent sand, silt and clay respectively. The soils are classified under the textural class clay loam. The bulk density, particle density, pH, electrical conductivity and cation exchange capacity of the soil were 1.38 Mg m^{-3}, 2.50 Mg m^{-3}, 7.60 dsm^{-1}, 0.86 dsm^{-1} and 22.4 c mol (p^+) kg^{-1} respectively. Organic carbon content of the soil was 3.9 g kg^{-1}. Available N,P and K content of the soil were 235.0, 14.0 and 170 kg ha^{-1} respectively, available sulphur content was 8.5 mg kg^{-1} and the exchangeable calcium, magnesium, potassium and sodium contents were 8.8, 8.2, 3.8 and 0.9 c mol (p^+) kg^{-1} respectively. The study was randomized block design with three replications and eight treatments. The treatments were T_1 - Absolute control, T_2 - Control (-K), T_3 - 12.5 kg of K_2O ha^{-1}, T_4 – 25 kg of K_2O ha^{-1}, T_5 – 37.5 kg of K_2O ha^{-1}, T_6 - 50 kg of K_2O ha^{-1}, T_7 – 62.5 kg of K_2O ha^{-1}, T_8 - 75 kg of K_2O ha^{-1}. The crops were harvested at maturity, well matured blackgram plants were harvested by cutting close to the ground using sickle and transported to threshing yard and sun dried for two days. Threshing was done by beating with sticks. The harvested crop of each plot was bundled separately. Grain and haulm yields were recorded plot wise and the yields were expressed in kg ha^{-1}. The economic parameters such as gross return and benefit cost ratio for all the treatments were worked out based on the prevailing market price. The net return was worked out for different treatments by subtracting the cost of cultivation from gross return. Benefit cost ratio invested was calculated by using the following formula.

$$\text{Benefit cost ratio} = \frac{\text{Gross return}}{\text{Total cost of cultivation}} \times 100$$

RESULTS AND DISCUSSION

YIELD OF BLACKGRAM

Grain yield and haulm yield of blackgram significantly increased due to potassium application. The maximum yield of blackgram recorded were obtained due to the application of 50 kg ha^{-1} of MOP resulted in significant higher grain yields over control. Grain and haulm yield increased due to the soil application of MOP 50 kg ha^{-1} (1080 kg $ha^{-1}$and 1483 kg ha^{-1}) which was 34 and 47% higher compared to absolute control (652 kg ha^{-1} and 806 kg ha^{-1}). It was clearly observed that yield increased with the optimum dose of potassium (50 kg ha^{-1}) and further reduced due to the over

dosage of potassium. Similar results were reported by Chaudhry and Mahmood (1999). Nasri *et al.* (2011) studied the effect of potassium on quality of mungbean and found that it has an important role in increasing grain yield through its effect on number of pods and number of grain per pod. The highest seed yield per hectare can be attributed to more number of pod per plant and number of seed per pod. Similar result was concluded by Samiullah and Khan, (2003).

ECONOMICS OF BLACKGRAM

Cost of production varied due to different dose of potash application. The lowest and highest production cost was absolute control and other all treatments respectively. Significant increase in gross income has been achieved by the usage of potassium in the forms of MOP. The treatments T_1 recorded the lowest gross income of 36843 Rs ha^{-1} and the treatment T_6 recorded the highest gross income of 49189 Rs ha^{-1}. However the treatment T_7 which recorded 48100Rs ha^{-1} was on par with treatment T_6 (49189Rs ha^{-1}).The other treatments T_3, T_4 and T_5 recorded gross income 42920 Rs ha^{-1}, 44830 Rs ha^{-1} and 46648Rs ha^{-1} which were found to be statistically significant. Benefit cost ratio, net income increased significantly by optimum potassium levels applied in the form of muriate of potash. Highest benefit cost ratio, net income was obtained due to the application of 50 kg MOP ha^{-1}. Benefit cost ratio, net income were 39%, 78% and 25%, 49% higher income compared to absolute control treatment and control(-K). Gross income significantly increased due to the application of 50 kg MOP ha^{-1}.Gross income which was 42% and 21% higher compared to absolute control and control (-K). Despite the addition input cost involved, the substantial yield increment obtained with this treatment @ 50 kg MOP ha^{-1}might have increased net income, Gross income and benefit cost ratio. The application of @ 50 kg MOP ha^{-1} had a less benefit cost ratio. High fertilizer cost of MOP might be the main reason. These finding are line with findings of Suryavanshi *et al. (*2008) and Suriyalashmi, (2013).

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Table 1: Effect of potassium on grain yield and haulm yield (kg ha^{-1}) of blackgram VBN-3

Treatments		Grain yield kg ha^{-1}	Haulm yield kg ha^{-1}
T_1	Absolute Control	652	806
T_2	Control (- K)	900	1154
T_3	12.5 kg of K_2O ha^{-1}	944	1197

T_4	25 kg of K_2O ha^{-1}	984	1280
T_5	37.5 kg of K_2O ha^{-1}	1030	1353
T_6	50 kg of K_2O ha^{-1}	1080	1483
T_7	62.5 kg of Kcl ha^{-1}	1060	1428
T_8	75 kg of K_2O ha^{-1}	1045	1400
SEd		9.4	26.5
CD(0.05)		19.6	55.3

Table 2: Effect of potassium on benefit cost ratio, net income (Rs ha^{-1}) and gross income (Rs ha^{-1}) of blackgram VBN-3

Treatments		Benefit cost ratio	Net income Rs ha^{-1}	Gross income Rs ha^{-1}
T_1	Absolute Control	0.87	6897	36843
T_2	control (- K)	1.27	9117	39855
T_3	12.5 kg of K_2O ha^{-1}	1.47	11106	42920
T_4	25 kg of K_2O ha^{-1}	1.79	13888	44830
T_5	37.5 kg of K_2O ha^{-1}	2.04	15953	46648
T_6	50 kg of K_2O ha^{-1}	3.18	18356	49189
T_7	62.5 kg of K_2O ha^{-1}	3.03	17146	48100
T_8	75 kg of K_2O ha^{-1}	2.59	16053	47059

Urea = Rs 6/kg
SSP = Rs 9/kg
MOP = Rs 14/kg

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RESEARCH ARTICLE

Effect of Zinc Enriched Organic Manures on the Groundnut Yield and Nutrients Availability in Coastal Saline Soil

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ABSTRACT

A field experiment entitled "Effect of zinc enriched organic manures on the groundnut yield and nutrients availability in coastal saline soil. The experiment was carried out in a farmer's field at Manalmedu coastal village near Chidambaram Taluk, Cuddalore district, Tamil Nadu during December– March 2020. The initial fertility status of experimental soil was pH – 8.37, EC- 1.73 d Sm^{-1}, organic carbon 2.7 g kg^{-1} and represented low status of zinc (0.71 mg kg^{-1}). The experiment encompassed nine treatments combinations viz., T_1 –Control/ RDF (100% NPK) on basis of STV), T_2 –RDF + FYM @ 12.5 t ha^{-1}, T_3 – RDF + Composted coirpith (CCP) @ 12.5 t ha^{-1} , T_4 – RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1}, T_5 – RDF + $ZnSO_4$ @ 25 kg ha^{-1} + Composted coirpith @ 12.5 t ha^{-1} , T_6 – RDF + zinc enriched farm yard manure (ZnEFYM) @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% foliar spray, T_7 – RDF + zinc enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% foliar spray, T_8 – RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % foliar spray and T_9 – RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % foliar spray. The experiment was laid out in a randomized block design (RBD) with three replications, using groundnut variety VRI 2 as test crop. The results revealed that application of recommended dose of fertilizer (17:34:54 NPK kg ha^{-1}) on the basis of soil test value (STV) + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % Foliar spray twice at pre flowering and flowering stages along with zinc enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} (T_9) recorded significantly higher effective groundnut yield and nutrients availability in coastal saline soil. This treatment was also recorded the highest pod yield of 2142 kg ha^{-1} and haulm yield of 2958 kg ha^{-1} as compared to control (100 per cent /NPK alone).

Keywords: Coastal saline soil, Zinc enriched FYM & CCP, Groundnut Yield, Nutrients availability.

INTRODUCTION

One of the most important oil seed crops growing in coastal areas is groundnut. It has a high protein content as well as vitamins E and C. Aside from the nutritional benefits of the crop, the high consumption of groundnut oil and by-products derived from the groundnut kernel assures the survival of low-income coastal farmers (Ramprosad Nandi *et al.,* 2020). Because of the inadequate physical constraints of coastal saline soils, all nutrients, including micronutrients, are lost by leaching. These two factors have a significant impact on coastal crop productivity. Nutrient management is one of the practises that can help to increase the growth and yield of groundnut. There are 147 million hectares of land are degraded, of which 10.78 million hectares are reported to be salt impacted and less productive, out of 329 million hectares land of total availability. Nearby 40,000 hectares of land in Tamil Nadu alone are impacted by salt. In Tamil Nadu, salt-affected soil covers 3.68 lakh ha, with coastal saline soils accounting for 1323 L ha and alkali soils accounting for 3.54 lakh ha. (Habbasha *et al*., 2013) It is produced on 5.26 million hectares in India and generates 9.67 million tonnes of food grains with an average pod yield of 1764 kg ha^{-1}. Groundnut is a prominent oilseed crop planted on 4.75 lakh hectares in Tamilnadu, with a production of 9.93 lakh million tonnes and a productivity of 2323 kg ha^{-1} (Agriculture statistics at a glance. 2020). Zinc is one of the most lacking nutrients in coastal soil, although it is required for peg formation, protein synthesis, and the establishment of chlorophyll pigment in groundnut. Organics such as FYM, CCP, and CCN are low-cost manures readily available in coastal areas; when applied to soil; operate as a carrier material for microbial development, a chelating agent, and an overall improvement in soil physical qualities. (Maharnor *et al*., 2018) Hence the present investigation was carried out to study zinc enriched organic manures on groundnut yield and nutrients availability in coastal saline soil.

MATERIALS AND METHODS

A field experiment was conducted in a farmer's field in Manalmedu coastal village from December to March 2020 to determine the influence of zinc-enriched organic manures on groundnut yield and nutrient availability in coastal saline soil. The various treatments included were, T_1 –Control (100% NPK/ RDF alone), T_2 –RDF + FYM @ 12.5 t ha^{-1} , T_3 – RDF + Composted coirpith (CCP) @ 12.5 t ha^{-1} , T_4 – RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1} , T_5 – RDF + $ZnSO_4$ @ 25 kg ha^{-1} + Composted coirpith @ 12.5 t ha^{-1} , T_6 – RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% foliar spray, T_7 – RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% foliar spray, T_8 – RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0 % foliar spray and T_9 – RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% + Humic acid @ 1.0% foliar spray. The experiment was laid out in a randomized block design (RBD), with three replications, using groundnut variety VRI 2. The experimental soil had sandy texture with pH- 8.37; EC- 1.73 d Sm^{-1}; organic carbon- 2.7 g $kg^{-1}$and Zinc status of 0.71 mg kg^{-1}. The alkaline $KMnO_4$–N; Olsen-P and NH_4OAc-K, were low (159.24 kg ha^{-1}), low (9.73 kg ha^{-1}) and medium (184.23 kg ha^{-1}) status, respectively. Calculated amount of inorganic fertilizer doses of Nitrogen (17 kg N ha^{-1}), Phosphorus (34 kg P_2O_5 ha^{-1}) and Potassium (54 kg K_2O ha^{-1}) were applied through urea, single super phosphate and muriate of potash, respectively. Calculated quantities of organic manures like FYM, composted coirpith (CCP), zinc enriched FYM (ZnEFYM) and zinc enriched composted coirpith (ZnECCP) were applied to the soil as per treatment schedule. Required quantities of zinc sulphate ($ZnSO_4$) as per the treatment schedule were incorporated. Foliar application of $ZnSO_4$ @ 0.5 per cent and Humic acid (HA) @ 1.0 per cent at Pre Flowering Stage (PFS) and at Flowering Stage (FS) was applied as per the treatment schedule. The biofertilizer *Rhizobium* @ 2 kg ha^{-1} was applied to all the experimental plots. The soil samples were collected at different critical stages of crop growth and analyzed for major (N, P and K) and micronutrients (Zn) content availability in soil as per the standard procedure (Jackson 1979). Upon different crucial stages of crop growth, crop growth parameters are recorded, as well as pod and haulm yield was recorded at harvest.

RESULTS AND DISCUSSION

Yield and yield characters of groundnut (Table 1)

Yield characters

The significant yield attributes were obtained with the application of RDF along with enriched organics as compared to control. The significantly higher number of pods plant^{-1} (29.36), 100 kernel weight (46.27), 100 pod weight (87.72) and shelling percentage (73.33) was recorded with the application of recommended dose of fertilizer + ZnECCP @ 6.25 t ha^{-1} through soil, as well as foliar sprays of $ZnSO_4$ @ 0.5 percent + Humic acid @ 1.0 percent twice at pre flowering and flowering stages (T_9). This was followed by the treatment T_8, (RDF + ZnEFYM @ 6.25 t ha^{-1} through soil application and foliar application of $ZnSO_4$ @ 0.5 percent + HA @ 1.0 percent), T_7, (RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5 percent), and T_6, (RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5 percent) by registering T_8 (25.83, 85.89, 44.92, 72.51), T_7 (27.61, 83.93, 43.49, 71.78), T_6 (26.64, 81.87, 42.11, 72.89) . The lowest yield characters of groundnut were recorded with treatment T_1 (RDF alone).

The application of 100 percent recommended dose of NPK + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through the soil, along with foliar sprays of ZnSO4@ 0.5 percent and Humic acid @ 1.0 percent twice increased the yield characters of groundnut by the direct influence of organic enrichment along with zinc. This could be because of the slow and steady release of nutrients by the enriched organics as the nutrient supply to sink at overall growth stages of groundnut. These results are in occurrence with the earlier findings of Chaithanya devi *et al*, (2003) and Karunakaran *et al*. (2010)

Yield

The influence of RDF + zinc enhanced organic manures, as well as foliar applications of $ZnSO_4$ and humic acid, on groundnut pod and haulm yield, was well demonstrated in this study. The highest pod yield (2142 kg ha^{-1}) and haulm yield (2958 kg ha^{-1}) were achieved under the nutrient-depleted coastal sandy soil with a combined application of recommended dose of fertilizer + ZnECCP @ 6.25 t ha^{-1} through soil, as well as foliar sprays of $ZnSO_4$ @ 0.5 percent + Humic acid @ 1.0 percent twice at pre flowering and flowering stages (T_9). This was followed by treatment T_8 - (RDF + ZnEFYM @ 6.25 t ha^{-1} through soil application and foliar application of $ZnSO_4$ @ 0.5 percent + HA @ 1.0 percent), T_7 - (RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5 percent), and T_6 - (RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5 percent) were the next treatments, with pod (2074, 2002, and 1927 kg ha^{-1}) and haulm (2859, 2757 and 2659 kg ha^{-1}) yield of groundnut, respectively. After that, the treatments were arranged in descending order like T_5>T_4>T_3 and T_2. These treatments were also statistically significant. Among the various Zn enriched treatments, the treatment (T_9), 100% recommended dose of NPK + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through soil + foliar spray of $ZnSO_4$ and Humic acid @ 0.5% twice recorded a pod and haulm yield of 2142 kg ha^{-1} and 2958 kg ha^{-1} which was 27.63 and 28.22 per cent increase over control or 100 per cent NPK alone. Groundnut yields were lower in the control treatment T_1, with lower pod (1550 kg ha^{-1}) and haulm (2123 kg ha^{-1}) yields.

The yield of groundnuts was done by incorporating a 100 percent recommended dose of NPK + Zn enriched composted coirpith (ZnECCP) @ 6.25 t ha^{-1} through the soil, along with foliar spray of ZnSO4@ 0.5 percent and Humic acid @ 1.0 percent twice. This could be because the use of Zn-enriched organics aided in the slow and consistent release of nutrients into the soil solution, which matched the groundnut's needed absorption pattern and so enhanced production of groundnut. This supports with earlier findings of Kadam *et al*. (2018). Furthermore, quick mineralization of N, P, and K from inorganic fertilizers, as well as consistent supply of these nutrients from Zn-enriched coirpith, may have supplied the crop's nutrient needs at critical phases. Furthermore, Zn involved in favourable effect on plant synthesis of nucleic acids resulting in increased pod yield due to increased availability of nutrients and photosynthates. These results are in agreement with those of Reddy *et al.,* (2011) and Shubhangi *et al.,* (2014). Foliar applications of $ZnSO_4$ and humic acids throughout the pre-flowering and flowering stages of crop growth were efficiently absorbed and translocated into the sink, resulting in a higher number of pods plant^{-1}.

Furthermore, increasing photosynthesis during growth stages may contribute to increased supply to the pods, resulting in improved seed setting and higher groundnut pod yield. The results are in sync with Jeetarwal *et al*. (2014); Kalita *et al*. (2015) and Gulam masthan vali *et al*., 2020

Nutrients availability in soil (Table 2)

Major nutrients availability (NPK)

Due to the application of zinc enriched organic manures, as well as foliar applications of $ZnSO_4$ and humic acid significantly influence the nutrient availability in soil by the adequate supply of nutrients in soil. The treatment T_9 (recommended dose of fertilizer + ZnECCP @ 6.25 t ha^{-1} through soil, as well as foliar sprays of $ZnSO_4$ @ 0.5 percent + Humic acid @ 1.0 percent twice at pre flowering and flowering stages) excelled the major nutrient availability of NPK 189.28, 21.62 and 265.38 kg ha^{-1} at harvest stage of groundnut over the rest of other treatments. This was followed by the treatments T_8 - (RDF + ZnEFYM @ 6.25 t ha^{-1} through soil and foliar application of $ZnSO_4$ @ 0.5 % + HA @ 1.0 %), T_7 - (RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5 percent), and T_6 - (RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5 percent) which registered (165.19, 164.41 and 163.59 kg ha^{-1}) of nitrogen, phosphorus (16.39, 15.61 and 14.79 kg ha^{-1}) and potassium (232.22, 226.24 and 220.42 kg ha^{-1}) in soil, respectively. Then coming under the other treatment follows with descending order like T_5>T_4>T_3 and T_2 with statistically significant. However, the less nutrient availability was noted in control T_1.

The nutrient availability in soil was increased due to the application of recommended dose of fertilizer + ZnECCP @ 6.25 t ha^{-1} through soil, as well as foliar sprays of $ZnSO_4$ @ 0.5 percent + Humic acid @ 1.0 percent twice at pre flowering and flowering stages. In line with the present study, Kabir *et al.,* (2013); Kamdi *et al.,* (2014) and Meena *et al.,* (2017) also reported similar results. Composted coir pith supplies the major nutrient in available form easily taken up by the plants. These findings are in agreement with Jahiruddin *et al*. (2014) and Arulrajsekaran *et al*. (2021).

Zinc availability (DTPA – Zn)

The combined application of RDF with Zn enriched organic manures and $ZnSO_4$ + HA foliar spray had a significant effect on groundnut zinc uptake at all the critical stages, particularly flowering, pod formation, and harvest. Among the various treatments, the highest Zn availability at flowering (1.72 g ha^{-1}), peg formation (1.50 kg ha^{-1}) and harvest (1.38 g ha^{-1}) stages was recorded with the application of RDF + ZnECCP @ 6.25 t ha^{-1} through soil and foliar spray of $ZnSO_4$ @ 0.5% + Humic acid @ 1.0% twice (T_9). This was followed by application of RDF + ZnEFYM @ 6.25 t ha^{-1} through soil and foliar application of $ZnSO_4$ + HA@ 0.5 per cent (T_8), application of RDF + ZnECCP @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% (T_7), and application of RDF + ZnEFYM @ 6.25 t ha^{-1} + $ZnSO_4$ @ 0.5% (T_6) which recorded nutrient availability of 1.36, 1.14 and 1.02 g ha^{-1} at flowering, peg formation and harvest stages of groundnut, respectively. This was followed by the treatments which received organics + $ZnSO_4$ along with recommended NPK (without Zn and HA foliar) applied treatments. The treatment T_5, (RDF + $ZnSO_4$ @ 25 kg ha^{-1} + CCP @ 12.5 t ha^{-1}) and T_4 (RDF + $ZnSO_4$ @ 25 kg ha^{-1} + FYM @ 12.5 t ha^{-1}) recorded a lowest Zn availability in soil as compared to above said treatments. This was followed by the treatments T_3 and T_2. These two treatments were also statistically significant. The control (100% NPK alone) treatment recorded the lowest Zn availability in soil at all the critical stages of groundnut.

The application of RDF + ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% and humic acid @ 1.0% twice significantly increased Zn availability in soil. These nutrients may have increased as a result of the addition of micronutrients along with Zn-enriched organics may have boosted the availability of these nutrients by increasing mineralization and chelation action, resulting in greater absorption and utilization for crops. The earlier reports of Nayek *et al.,* (2015) and Prashantha *et al.,* (2019) support the present findings. Further, increased availability of Zn in soil were noticed in the treatment supplied with ZnECCP @ 6.25 t ha^{-1} through soil along with foliar spray of $ZnSO_4$ @ 0.5% and humic acid @ 1.0% twice. These findings corroborate the earlier results of Kulhare *et al*., (2014) and Jat *et al*., (2015).

CONCLUSIONS

The results of this study clearly indicated that the application of RDF + ZnECCP @ 6.25 t ha^{-1} through soil along with foliar sprays of $ZnSO_4$ @ 0.5 percent and Humic acid @ 1.0 percent twice at the pre flowering and flowering stages would be beneficial for increasing groundnut growth and yield, as well as nutrient availability status in coastal saline soil.

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Table 1. Effect of Zn enriched organics on the yield characters and yield of groundnut in coastal saline soil

Treatments	Number of pods plant^{-1}	100 Pod Weight(g)	100 kernel weight (g)	Shelling (%)	Yield (kg ha^{-1})	
					pod	haulm
T_1	22.27	72.36	35.34	66.86	1550	2123
T_2	23.08	74.33	36.63	67.78	1632	2225
T_3	23.95	76.15	37.95	68.64	1710	2323
T_4	24.86	78.08	39.34	69.36	1782	2438
T_5	25.81	79.94	40.68	70.15	1856	2549
T_6	26.64	81.87	42.11	70.89	1927	2659
T_7	27.61	83.93	43.49	71.78	2002	2757
T_8	28.53	85.89	44.92	72.51	2074	2859
T_9	29.36	87.72	46.27	73.33	2142	2958
SE_D	0.37	0.85	0.61	0.31	23.05	35.25
CD (p=0.05)	0.75	1.72	1.23	0.64	65.15	94.09

Table 2. Effect of Zn enriched organics on the NPK & Zn availability in coastal saline soil

Treatments	Nitrogen (kg ha^{-1})			Phosphorus (kg ha^{-1})			Potassium (kg ha^{-1})			DTPA- Zn (mg kg^{-1})		
	FS	PFS	HS	FS	PFS	HS	FS	PFS	HS	FS	PFS	HS
T_1	165.47	161.97	158.63	11.81	10.2	9.83	231.29	209.96	188.25	0.77	0.67	0.64
T_2	168.75	163.25	159.91	13.09	12.17	11.11	235.85	215.52	194.21	0.92	0.76	0.71
T_3	172.07	164.57	160.83	14.41	14.12	12.03	240.17	220.84	201.02	1.03	0.84	0.79
T_4	175.19	167.69	161.75	15.53	16.04	12.95	244.29	225.96	207.54	1.15	0.95	0.85
T_5	178.54	169.04	162.60	16.88	16.85	13.8	248.64	231.65	214.43	1.25	1.04	0.92
T_6	181.83	170.33	163.59	18.17	17.67	14.79	252.93	236.94	220.42	1.36	1.14	1.02
T_7	183.95	171.45	164.41	19.29	18.49	15.61	257.05	242.06	226.24	1.49	1.27	1.15
T_8	187.13	172.63	165.19	20.47	19.33	16.39	261.23	247.24	232.22	1.58	1.36	1.24
T_9	189.28	174.78	167.34	21.62	20.09	18.54	265.38	252.39	238.17	1.72	1.50	1.38
SE_D	1.01	0.53	0.32	0.53	0.39	0.35	1.99	1.89	1.81	0.041	0.033	0.026
CD (p=0.05)	2.05	1.07	0.64	1.07	0.79	0.71	4.02	3.82	3.65	0.092	0.075	0.057

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RESEARCH ARTICLE

Finger Knuckle Print Recognition using Long Short-Term Memory

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ABSTRACT

Biometric technology has received a lot of attention in recent years. One of the most prevalent biometric traits is the finger-knuckle print (FKP). This work aims to create an autonomous deep learning framework for FKP authentication systems with a high recognition rate and rapid authentication. The proposed deep learning models are evaluated using MATLAB 2021b and WEKA tool version (3.6.9) are used to train and test using the extracted features, and to classify the obtained deep features, SVM & Softmax classifiers are used. A hybrid model is proposed combined ResNet & LSTM. The combined ResNet + LSTM architecture, which attains a precision of 98.7% and a recall of 98.8% and based on the F1-Score, the model's total performance is 99.1%. The designed hybrid method can improve the recognition rate and the recognition speed for Finger-Knuckle-Print based biometric systems.

Keywords: Biometrics, Finger Knuckle Print, Authentication, Identification

INTRODUCTION

Automated identification systems are vital for security and privacy in the digital age [1]. Biometrics is a Greek term that meaning to measure life [2]. Biometrics measures human traits to verify or identify a person. A biometrics system automates person recognition. In IT, biometrics is used for identity access management and access control. It's also used to identify undercover group members. A person identify may be verified or rejected [1]. This is authenticating or verifying. Authentication establishes a user's legitimacy by comparing claimed and real credentials. Authentication entails matching the database or identifying elements. The authentication matching result is "Yes" or

"No," where "Yes" implies the individual's claim can be confirmed and "No" means it cannot. Logical or physical access to a defined region requires authentication. Identification, also known as Recognition, refers to determining a subject identify from a collection of known identities (closed identification problem) or otherwise (open identification system) [1]. In other words, identification compares a person's templates to those in a database. ID involves a one-to-many database search. The identification search yields a list of probable matches with the most similarities or exceeding a given threshold. Usually used for physical access or law enforcement. Humans prefer to use "what they know" for authentication and identification, such passwords or PINs. Passwords may be exchanged, stolen, or guessed. So, passwords should be a minimum length, in alphanumeric format, restricted in time, distinct in each application, and regularly updated. These rules make it hard to remember passwords. People scrawl passwords on keyboards and displays. People utilise "what they have," such as identifying goods, to authenticate and identify others. ID card, credit card, smart card, and signature stamp are ID objects. Unauthorized people may take and use IDs for illicit activity. Biometric "what you are" is presented to solve these flaws. Biometric technologies can be compared using parameters such as universality, uniqueness, permanence, collectability, performance, acceptability and circumvention.

Universality
Every individual using the biometric system should have the same biometric characteristics.

Uniqueness
The biometric features must be distinctive from one individual to another in the biometric system.

Permanence
The biometric features should be resistance to aging. It should not change much across a period of time.

Collectability
The biometric features should be easy to collect, using acquisition devices or sensors, and measurable.

Performance
How the biometric system performs in term of accuracy, speed and robustness.

Acceptability
How the public or private users receive the biometric technology and willingness of using the biometric system.

Circumvention
How easy the biometric features being imitated, substituted or mimicked. For example, fake finger in fingerprint identification.

Hand-based Authentication Systems
Models that detect fingerprints [3–7], palm prints, hand geometry, hand shape, and hand veins [8, 9] are examples of hand-based identification systems. Hand-based systems have been in the forefront of attention for a significant amount of time [10]. The reliability of the system's attributes, which contribute to its stability, acceptability, simplicity, and robustness, is largely responsible for the success of the systems [11]. Hand-held technology is used for a variety of applications today, including security, by a significant number of businesses, industries, and government organisations [12].

Finger-Knuckle Print
In addition to be a kind of biometric information, the Finger-Knuckle Print (FKP) [13] may also be used in reliable authentication systems [14]. [13] Images include information about an individual's finger knuckle lines and textures, which may become a distinctive anatomical trait and be used to identify a person. This is a hand-based characteristic that can be used to identify a person. In its most basic sense, the term "finger knuckle surface" refers to the skin

patterns that may be seen on the back, or dorsal, side of a human hand. The human hand has three phalangeal joints in each finger dorsal region: I the Metacarpo Inter Phalangeal (MIP) joint, which connects the finger to the surface of the hand; ii) the Proximal Inter Phalangeal (PIP) joint, which is formed in the middle surface of the finger; and iii) the Distal Inter Phalangeal (DIP) joint, which is present in the tip surface of the finger dor This bending of the skin at the external surface of the finger dorsal region is caused by these joints, which also contribute to the formation of dermal patterns that include curved curves, contours, and wrinkles. Finger knuckle print (FKP) and the skin pattern created via the finger area dorsal joints PIP and DIP refer to the Finger Back Knuckles Surface [FBKS], as shown in Figure 1. The PIP joint design consists of a finger knuckle pattern in the dorsal part of the finger area.

LITERATURE REVIEW

The finger knuckle print is a global, one-of-a-kind, and persistent biometric pattern that is utilized for extremely exact personal identification. Recent FKP research has focused on robust feature extraction, contactless/unconstrained acquisition, and fusion techniques [15]. However, the literature includes limited work. FKP authentication using Gabor Filter phase congruency [16]. New FKP feature extraction software. Local direction, phase, and phase congruency were studied. They calculated all characteristics using phase congruency [17]. Gabor-SVM for FKP [18].A unique FKP biometric system that blends EPH and textural properties (SSTF). GA found the best characteristics. PolyU [19]-tested. Singh and Kant designed a multimodal biometric system for person authentication based on FKP and iris characteristics [20]. Finger joint biometric identification system. This authentication uses deep learning. CNN obtains image attributes. Backpropagation, random gradient descent, and minibatch learning train CNN [21].Using PCA to train two filter banks, binary hashing and block histograms cluster feature vectors. Last is linear multiclass SVM classification (SVM). They also studied a score-fusion-based multimodal biometric system [22].A CNN end-to-end FKP paradigm. The dataset Poly-U FKP is used to evaluate the recommended model, yielding 99.83% (0.76 and 99.18%, respectively) recognition accuracy [23]. This model features two connected and three convolutional layers despite its basic data augmentation technique and few trainable parameters. Chalabi et al. used PCANet-SVM to combine finger knuckle scores [24].Hamidi et al. used deep convolutional neural networks to extract information from Finger-Knuckle-Print images. Matching score level fusion works well for unimodal and multimodal identification [25]. Fei et al. produced a new finger knuckle direction vector.

They introduced a finger knuckle picture learning approach. The suggested method outperforms previous Finger knuckle image recognition techniques [26].Deep learning models power computer vision. ResNet [27] deepens CNNs and prevents learning deterioration by using the identity block. MobileNet [28] also links bottleneck layers with inverted residual. This tiny type is portable. CNN's ShuffleNet is mobile-friendly. This architecture's low computational power is versatile. This CNN's processing is reduced via channel shuffling and pointwise group convolution. EfficientNet [30] enhanced CNN performance. EfficientNet recommends increasing CNN depth, layers, breadth, filters, and input image resolution. This architecture offers depth, width, and resolution scaling versions b0 through b7.FKP is often used with other biometrics to increase security. Despite the many FKP identification techniques, none is perfect; each has computer vision and machine learning-induced limitations. Deep learning on FKP images needs work. Biometrics include fingerprint, palm print, hand geometry, and hand vein. Biometric identifier FKP. Since people hold goods with their hands, FKP is hard to abrade. Non-contact FKP collection is more popular with users. FKP is a useful future personal identification technology.

METHODOLOGY

A generalized block diagram of the FPK authentication system using deep learning model is presented in Figure 2. In order to extract features, the CNN architectures which are pretrained on the Image Net dataset are used in this work. For feature extraction and training, MATLAB 2021b and WEKA tool are used. The Weka tool version (3.6.9) is used to train and test using the extracted features, and to classify the obtained deep features SVM & SOFTMAX Classifiers

are used. The proposed work is conducted on Intel(R) Core (TM) i7-6700 CPU at 3.40 GHz with 8 GB memory. All the WEKA methods are used with their default parameters. The dataset is divided randomly in the ratio of 80% training and 20% testing ratio. The training and the testing images are fed into the proposed models and the features are extracted. At last the deep learning models will identify the FPK images.

Database

To achieve the objectives of the study, especially as it employs deep learning, the Hong Kong Polytechnic University contactless finger knuckle images database (Version 1.0) is used. Which is contributed from the male and female volunteers. This database has been largely acquired in The Hong Kong Polytechnic University campus and IIT Delhi Campus during 2006-2013 using a contactless setup that simply uses a handheld camera. This database has 2515 finger dorsal images from the middle finger of 503 subjects, all the images are in bitmap (*.bmp) format. In this dataset about 88% of the subjects are younger than 30 years. This database also provides two session finger knuckle images acquired after very long interval (4 to 7 years) to ascertain stability of knuckle crease and curved lines. Figure 3 shows sample images of the used dataset.

Performance Evaluation Metrics

To evaluate the performances of the implemented model, we have used different performance measure metrics such as accuracy, precision, recall, specificity, and F1-score. To evaluate these metrics, we need several indices such as True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) are the four labels derived from the confusion matrix as shown in figure 3.3. TP is the correctly classified images in a particular category. FP is the number of wrongly classified images, whereas TN is the sum of the correctly classified images in all other categories and FN is the number of misclassified images from the relevant category.

Precision

The precision is calculated as the ratio between the number of Positive samples correctly classified to the total number of samples classified as Positive (either correctly or incorrectly). The precision measures the model's accuracy in classifying a sample as positive.

$$Precision = \frac{TP}{TP+FP} \quad (3.1)$$

Recall

The recall is calculated as the ratio between the number of Positive samples correctly classified as Positive to the total number of Positive samples. The recall measures the model's ability to detect Positive samples. The higher the recall, the more positive samples detected.

$$Recall = \frac{TP}{TP+FN} \quad (3.2)$$

F1 Score

The F1 score is defined as the harmonic mean of precision and recall. The goal of the F1 score is to combine the precision and recall metrics into a single metric. At the same time, the F1 score has been designed to work well on imbalanced data.

$$F1 = 2\frac{Presision*Recall}{Presision+Recall} \quad (3.3)$$

LONG SHORT-TERM MEMORY

Recurrent neural networks construct a directed graph along a succession of variables (e.g., a temporal sequence). Recurrent connections enable modelling of a variable's present and prior states. The RNN-based technique is popular because it can anticipate sequential data, such as in language translation or action recognition. Improved RNN models, such as LSTMs or GRUs, allow training on extended sequences, overcoming vanishing gradients. In this chapter, RNN uses LSTM Units to highlight FKP characteristics. This helps the model gain biometric identifying qualities. First, the ResNet CNN, which outperforms other CNNs on FKP authentication, is utilised as a visual

feature extractor: an FKP picture is encoded as CNN features (i.e., a tensor of feature maps at a given output of a chosen convolutional layer). These CNN features are a smaller picture of activations with as many channels as convolution layer filters. This new picture is divided into equal-sized sub-parts to achieve local activations in numerous places. These new local CNN characteristics may be utilised to generate a sequence and feed an LSTM-based RNN to find essential CNN components. It increases each subpart's effective pixel neighbourhood and optimises information acquisition across CNN subparts. Optimization minimises prediction error.

RESULTS AND DISCUSSION

In this chapter the hybrid ResNet + LSTM is implemented in order to compare and quantify the performance with the proposed model for the efficient FKP authentication. To evaluate the performances of the implemented model, the different performance metrics such as accuracy, precision, recall, and f1-score are used. The performances of the combined ResNet & LSTM are applied on the database. The experimental findings of the suggested frameworks are presented and discussed. Based on Table 1 it is observed that ResNet & LSTM achieves a Precision of 98.7% & Recall of 98.8% and based on the F1-Score, the model's total performance is 99.1%.

CONCLUSION

Biometric technology has received a lot of attention in recent years. One of the most prevalent biometric traits is the finger-knuckle print (FKP). Because the dorsal region of the finger is not exposed to surfaces, FKP would be a dependable and trustworthy biometric. A hybrid model is proposed combined ResNet & LSTM. The combined ResNet + LSTM architecture which attains a Precision of 98.7% & Recall of 98.8% and based on the F1-Score, the model's total performance is 99.1%. The designed method can improve the recognition rate and the recognition speed for Finger-Knuckle-Print based biometric systems.

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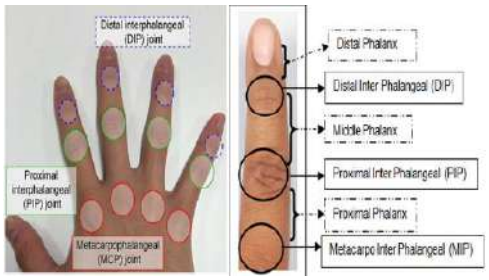
Table 1 Results of ResNet & LSTM

Precision	Recall	F1 Score
0.987	0.98.8	0.992



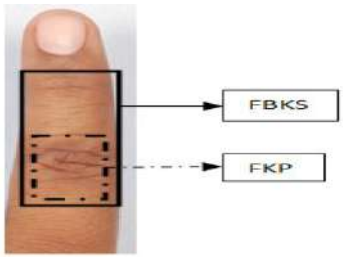


Figure 1 Physiological Characteristics of Finger Knuckle Surface

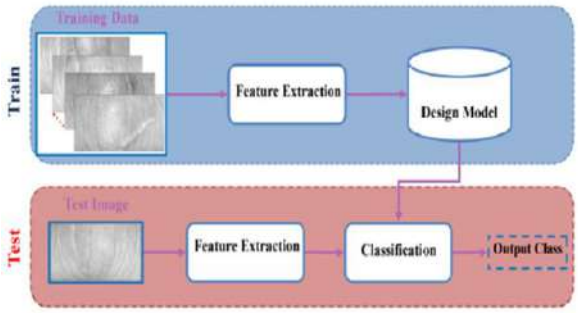


Figure 2 Block diagram of FPK authentication system.

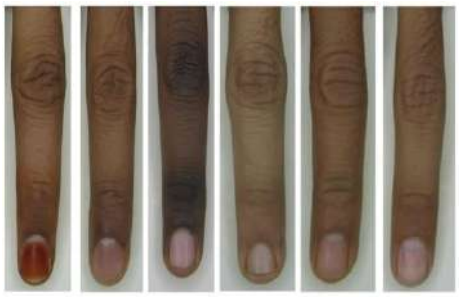


Figure 3 Sample images from the database.

Figure 4 Confusion Matrix

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RESEARCH ARTICLE

Air Pollution Tolerance Index of Plants Living in High Traffic Areas of Tiruchirappalli City

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ABSTRACT

In the present study, Air pollution Tolerance Index (APTI) was calculated for various plant species growing in two different locations Tiruchirappalli city (study side) and Bharathidasan University Campus (Control side) of Tiruchirappalli, Tamil Nadu. Naturally plant species can act as for air pollutants; they modify their biochemical characteristics under stress. An APTI score of sensitive ≤11, intermediate 12–16, and tolerant ≥17 classifies the tree species as, and towards air pollution respectively. Sensitive and tolerant plant species can be identified by evaluating their air pollution tolerance index (APTI).The plant leaf samples were collected from seven plant species. Biochemical estimations were analyzed, such as relative water content, leaf extract pH, total chlorophyll content, and ascorbic acid content, were used to compute the air pollution tolerance index values. *Azadirachta indica* (Neem), *Polyalthia longifolia* (Ashoka), *Pongamia Pinnata* (Pongam), *Peltophorum pterocarpum* (Yellow flame tree), *Tamarindus indica* (Tamarind), *Delonix regia* (Peacock flower tree), *Senna auriculata* (Avaram) The air pollution tolerance index was calculated from obtained values. On the basis of air pollution tolerance index values for above-mentioned seven plants, *Azadirachta indica* established the significant level of APTI most of the tested plants grown at the roadside plants had a higher air pollution tolerance index than those grown on university campus.

Keywords: Pollution Tolerance, Leaf extract pH, Chlorophyll content, Relative water content, Ascorbic acid content.

INTRODUCTION

Environmental pollution is a major concern in urban areas, all over the world due to rapid urban growth, particularly in developing countries, leads vehicular pollutants and gaseous pollutants (Joshi and Swami 2009). These pollutants mainly arise from anthropogenic activities such as Industrialization, Urbanization and Transportation (Odilora et al., 2006). Most of the cities, transportation is the main source of the air pollution which contaminates air everywhere (Thawale et al., 2011). The Society of Indian Automobile Manufactures (SIAM, 2012) reported that in 2016-17 India sold 21 million vehicles and after two years this number has scaled up to 26 million at 2018-19. Moreover, the unplanned urban development is a major reason for the increase in the pollution level in urban areas (Jayanthi and Krishnamoorthy, 2006). In nature, plants play a major role in monitoring and maintaining the ecological balance (Escobedo et al., 2008).Plants are an integral part of our ecosystem, trees and shrubs, being long-lived components of the landscape, play a significant role in improving the environmental conditions. Plant leaves, being abundant and having a large surface area, are major air pollutant receptors (Chauhan 2010; Panigrahi et al., 2014). In addition, plants may absorb particulate air pollutants through their leaves (Setala et al., 2013). The phytoremediation effects of plants on removing toxic pollutants and improving air quality (Teiri et al., 2018). The response of plants to pollutant absorption can be used as an economical and sustainable tool to monitor air quality (Rai et al., 2013). Plant's ability to tolerate air pollutants depends on its biochemical, physiological and morphological characteristics (Singh SN, Verma. A 2007)Trees and shrubs are more affected by pollutants because plants exhibit different responses and absorption/resistance to various pollutants. Air pollution tolerance index (APTI) is an important parameter to screen out different plants to determine their susceptibility or tolerance to different pollutants (Sabri et al., 2015; Krishnaveni et al., 2014). This index is calculated by estimating leaf relative water content, pH of leaf extract, ascorbic acid and leaf chlorophyll contents (Rao 1977, Klumpp et al., 2000, Hoque et al., 2007, Govindaraju et al., 2012). Species with lower APTI act as bio-indicators of air pollution, while those with higher APTI values are tolerant to pollutants (Ogunkunle et al., 2015).

MATERIALS AND METHODS

Study area

Tiruchirappalli City is the most important region in the State. The city is located at the Central part and fourth largest city in Tamil Nadu, India. In Tiruchirappalli city the river cauvery splits into two branches, one is northern branch being called the Coleroon (Kolidam) and another one is Southern branch is called river cauvery. The Cauvery River is an important river in the city. The city spread over an area of 167square Kilometres and geographical limit between 10.8050°N to 78.6856°E with total population of above 1,182,000 in 2021survey data. The average annual rainfall is around 823mm. Average maximum temperature in the area is around 40°C, with the minimum settling close to 28°C. Tiruchirappalli city is the sampling site and Bharathidasan University is a Control site.

Plant leaf Sample collection

The study was conducted in Tiruchirappalli city during the period from 2020 and2021. Samples of uniform size and age from healthy growing plant species were collected from two locations: study site and control site. Totally Seven plant species are selected for the present study are *Azadirachta indica, Polyalthia longifolia, Pongamia Pinnata, Peltophorum pterocarpum, Tamarindus indica, Delonix regia, Senna auriculata*, were collected. The fresh leaves were picked and carry using transparent polythene bags were put into the liquid nitrogen container. The fresh weight of leaves was measured immediately. The leaf samples were maintained at -20^{o}C until further analysis.

Biochemical analysis

The plant samples were evaluated for four biochemical parameters, including leaf extract pH (pH), Relative water content (RWC), Total chlorophyll content (TLC) and Ascorbic acid content (AAC) to calculate the APTI.

Leaf extract pH

Leaf extract pH was determined by the method of Agbaire and Esiefarienrhe 2009; Tsega and Prasad, 2014. Two grams of leaf samples from each plant species was homogenized in 20 ml of distilled water, left for 20 minutes, the mixture was centrifuged and supernatant was collected after pH was measured using a pH meter.

Relative Water Content (RWC)

Relative water content was determined by Schlemmer 2005; Agbaire and Esiefarienrhe 2009. Fresh leaf samples were weighed to record fresh weight, and immersed in distilled water for 4 hrs in the refrigerator, blotted dry with filter paper Whatman No. 1, Sigma Aldrich, weighed again to measure turgid weight. Afterwards, leaves were placed in the oven at 70 °C for 48 hrs and reweighed to the dry weight.

Calculated using the following formula:

$$RWC=[(FW-DW)/ (TW – DW)]\times100$$

Where, FW = fresh weight of the leaf
TW = turgid weight of the leaf after immersing into water overnight
DW = dry weight of the leaf.

Total Chlorophyll

Total chlorophyll content determined by the method of Arnon 1949 and Wellburn 1994. 0.5 g of leaf material from each sample was ground and diluted to 10 mL in distilled water. A subsample of 2.5 mL was mixed with 10 ml of 80% acetone and centrifuged at 2500 rpm for 5 minutes after filtered. Supernatant was collected and the pellet was re-extract twice with same volume of solvent. This step is repeated three to four times the pellet was changed into white in colour. The supernatants were collected in a container and the absorbance was measured at 645 nm and 663 nm. Using UV- Visible Spectrophotometer.

Calculations by using the formula:

$$\text{Chlorophyll a (D663)} = 12.7D663 – 2.69D645\times V/1000\ W\ mg/g$$

$$\text{Chlorophyll b (D645)} = 22.9D645 – 4.68D663 \times V/1000\ W\ mg/g.$$

Ascorbic Acid Content

Ascorbic acid content of fresh leaf samples was determined by the method used by Bajaj and Kaur1981; Campos 2009. 1g of collected fresh leaf samples was put in a test tube and mixed with 2 ml of oxalic acid EDTA extracting solution followed by mixing with 1 ml of acetic acid, 1 ml of 5% tetraoxo sulphate acid, and 2 ml of ammonium molybdate. Finally, 3 ml of water was added to the test tube and left for 15 min at room temperature. The concentration of ascorbic acid in the samples was then calculated from a standard ascorbic acid curve. For this, 0.1 to 0.6 ml aliquots of standard ascorbic acid solution were taken in test tubes and chemicals were added as before. After the incubation period absorbance was measured at 760 nm and a standard graph was prepared.

Air Pollution Tolerance Index (APTI)

Leaf extract pH, Relative water content of the leaves, Total chlorophyll, and Ascorbic acid was determined by the method used by (Singh and Rao 1983). For the calculation of Air pollution tolerance index (APTI) Nayak et al., 2015; Pathak et al., 2011.

$$APTI=A\ (T+P) + R/10$$

Where: A = Ascorbic acid (mg/ g)
T = Total chlorophyll (mg/g fresh weight)
P = pH of the leaf extract
R = Relative water content of the leaves (%)

RESULTS AND DISCUSSION

pH of Leaf extracts

The pH extracts of leaf control are several factors in tolerant plants. pH signals as an indicator of the sensitivity for the air pollution. Lower pH plants are susceptible, while plants around pH 7 are more tolerant. Acidic air pollutants can reduce leaf extract pH (Girish et al., 2017). Overall plants showed pH (fig 2). Maximum ranged from control *P. pterocarpum* (6.08), *A. indica* (5.86), and the lowest leaf extract pH *T. indica* (3.10), *P. pinnata* (4.01). The study side plants species had almost natural pH values of level of maximum leaf extract pH was found in *pongamia pinnata* (6.32) in unban study site, whereas, the minimum was observed in *Tamarindus indica* (3.10) in control site. pH act as he occurrence of detoxification processes in plants, its necessary for tolerance (Thawale et al., 2011). Low pH values are an indication of sensitivity of plant species to air pollutants, high pH could provide tolerance to pollutants (Govindaraju et al., 2012).Photosynthesis efficiency of plants is pH dependent and at lower acidic pH photosynthesis efficiency of plants gets reduced (Yan-ju and Hui 2008).

Relative water content

Urban study site has higher air pollutant compared to control site by evaluation of relativewater content of selected plant samples. The highest relative water content in leaves of the plant species at the pollution site was observed at maximum level in *A. indica* 92.19% and minimum level in *P. pinnata* 57.42%. However, in control, the relative water content of leaves of the plant species varied from a maximum level in *T. indica* 75.02% and minimum level of *P. pterocarpum* shown in Fig 1.Transpiration rate in leaves is affected by pollutants and it reduce the relative water content of plant species (Swami et al., 2004).The plant as associated with protoplasmic permeability in cells and causes loss of water content and dissolved nutrients. (Lakshmi et al., 2009). The high level of water content present in plant body maintains the different physiological balances under stress conditions of the pollutions. Increased level of pollution increased the permeability of cell and dissolved nutrients increasing of risk of the early senescence. (Dipti Karmakar et al., 2021)

Total Chlorophyll Content (Tch)

The total chlorophyll content in the plant species of control and study site was compared. It was observed that total chlorophyll content decreased at the study site. The chlorophyll content in leaves samples of selected plant species collected from the pollution site was low, when compared with the samples from the control site. Whereas, at the study site, maximum levels were seen in *P.longifolia* (2.70) and minimum levels in *P.pterocarpum* (1.02).The control site maximum level of total chlorophyll content was found in *P.pinnata* (4.89) and minimum level in *T.indica* (1.20). It is well known that air pollutants are degraded in the photosynthetic pigments of plant leaves. Chlorophyll content of plants signifies its photosynthetic activity as well as the plant growth and plant development of biomass. The plant are able to maintain the cchlorophyll content in the polluted condition is known to be tolerant species (Sing and verma. 2007). Total chlorophyll content (Tch) is varies from a species to species and leaf age also the pollution level as well as the other abiotic and biotic conditions(Katiyar and Dubey 2001; Abida and Harikrishna 2010; Rai and Panda 2015).The plants which contain higher chlorophyll or try to increase it, kept the chlorophyll at constant level are suggested as tolerant to pollutants (Joshi et al. 1993).

Ascorbic acid contents

The plant species growing at the study site, *P. longifolia, A. indica* and *S. auriculata* had the highest ascorbic acid contents of 4.20, 3.86 and 3.18 mg/g. While, *T.indica, P.pterocarpum, P.pinnata*and *D.regia* had the lowest ascorbic acid contents in their leaves, 1.50, 1.52, 2.13 and 2.20 mg/grespectively. The plants growing in control site, *D.regia*is exceededthe level than all other species with 2.50 mg/g ascorbic acid contents, while *P.longifolia A.indica* and *P.pinnata* had the lowest ascorbic acid contents in their leaves, 0.4, 0.8 and 0.70 mg/g. Ascorbic acid is a strong reductant and it activates many physiological and defense mechanisms in plants. Its reducing power is directly proportional to its concentration (Raza and Murthy, 1988; Agbaire and Esiefarienrhe, 2009). Increased level of ascorbic acid in oliar tissue increases the tolerance against air pollution and also other environmental stresses (Nadgorska –Sochaet al.,

2017)However, it's reducing activity of pH dependent on being more at higher pH, because high pH level may increase the efficiency of conversion of the hexose sugar to ascorbic acid and is related to the tolerance to pollution (Liu and Ding, 2008)Ascorbic acid is an antioxidant activate commonly found in healthy plants that depicts its resistance to air pollutions. (Pathak et al 2011). In Previous studies, the ascorbic acid reduces Reactive Oxygen Species (ROS) concentration in leaves. The higher ascorbic acid content of a plant is the indicator of tolerance against SO_2 pollution (Jyothi and Jaya 2010; Varshney and Varshney 1984).

Air Pollution Tolerance Index (APTI)

The APTI values of selected plants for the pollution site and control are represented in Fig 5. The highest APTI values scored by pollution site plants A.indica (12.5) P.longifolia (11.2), S. auriculata(9.7), D. regia (9.3), T. indica (8.7), P. pterocarpum (7.6) and the lowest APTI value was observed in P. pinnata (7.5). The control site A.indica (12.5) P.longifolia (11.2), S. auriculata(9.7), D. regia (9.3), T. indica (8.7), P. pterocarpum (7.6) High APTI values performance recommended a tree species as tolerant variety, while the lower values of these parameters plants as sensitive to air pollution.APTI is an index through which a plant could be judged whether it is tolerant or sensitive to air pollution (Singh et al., 1991). A higher index value represents tolerance in air pollution and the lower values of it indicate sensitivity of plants (Joshi and Swami 2007).

CONCLUSION

APTI values calculated for the seven plants it can be concluded that *A.indica* is found to be a suitable air pollutant tolerant plant species with its observed biochemical characteristics against the air pollution stress. Also, significant increase in APTI values in plants growing at polluted site. The level of air pollution tolerance may vary from species to species. Hence, this study suggested that the plants such as *Azadirachta indica, Polyalthia longifolia, Pongamia Pinnata, Peltophorum pterocarpum, Tamarindus indica, Delonix regia, Senna auriculata*can be used as a bio-indicator of air pollution monitoring practices.

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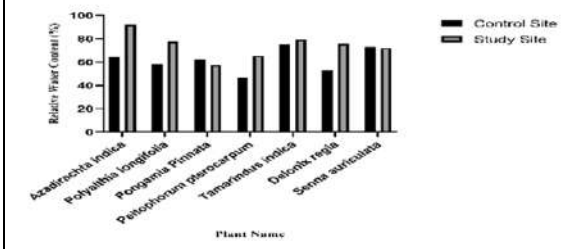

Fig.1. Relative Water Content in leaves (%)

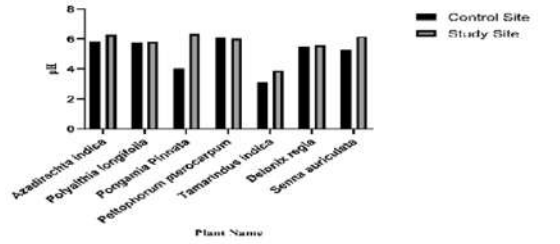

Fig.2. Leaf extract pH in leaves

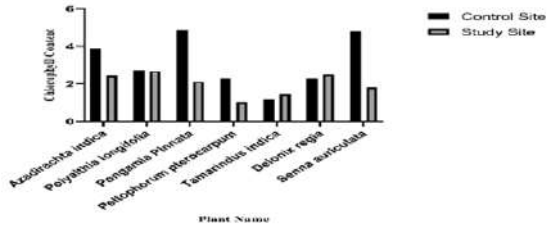

Fig.3.Total Chlorophyll Content in leaves (mg/g)

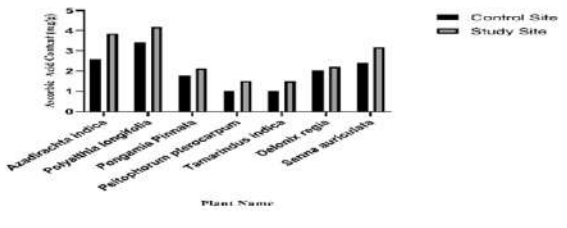

Fig.4.Ascorbic acid in leaves (mg/g)

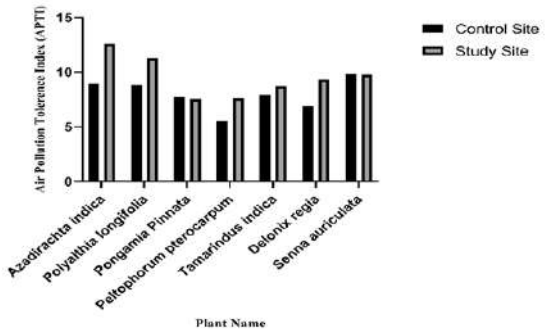

Fig.5.Air Pollution Tolerance index (APTI)of selected plant Species in study site and control site

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RESEARCH ARTICLE

Attitude of Paddy Growers towards Climate Change on Agriculture in Cauvery Delta Zone of Tamil Nadu

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ABSTRACT

Agriculture plays a dominant role in the Indian economy and provides food and livelihood security of the Indian population. Climate is the primary determinant of agricultural productivity. Climate change is expected to impact on agricultural productivity and shifting cropping patterns. A lower agricultural production and productivity due to climate change has implication for food prices, which in turn affect the livelihood and food security of the farmers. Under the circumstances climate resilient practices according to weather forecast based agro advisories are more useful to reduce vulnerability and improve adoptability of agriculture to climate change. Keeping in view of the above points, the present study attitude of paddy growers towards climate change on agriculture was designed and undertaken.In this study was conducted in Cauvery Delta Zone of Tamil Nadu. A sample size of 240 paddy growers were selected randomly from the selected villages. A well-structured interview schedule has been used for collecting responses. The results of the study revealed that more than half of respondents(59.58 %) were found to possess high level of attitude towards climate change. Further, the respondents statement wise attitude towards climate change viz., the serious problem and action to be taken on the livelihood of farmers was ranked first (4.79MS) and climate change affects the food security, income and employment of villagers were ranked second (4.76MS). The findings of this research may help extension agencies for creating awareness and better adaptation strategies climate change.

Keywords: Paddy Growers, Climate Change, Attitude, Agriculture, Cauvery Delta Zone (CDZ).

INTRODUCTION

Agriculture is the backbone of economy in most of the developing countries. In addition to food and raw material, agriculture also provides employment opportunities to large section of the population. Agriculture and climate are mutually dependent (Balu and Kavaskar, 2021). Climate change has been defined by the Intergovernmental Panel on Climate Change, IPCC (2007) as statistically significant variations in climate that persist for an extended period, typically decades or longer. Climate change directly affects agriculture production as this sector is inherently sensitive to climatic conditions and is one of the most vulnerable sectors at the risk and impact of global climate change ((Parry et al. 2005). India is a large country with a diverse climate, diverse seasons, diverse crops and diverse farming systems. There is a high dependency of agriculture on monsoon rains and a close link exists between climate and water resources. The impacts of climate change are multidimensional and global. Countries like India are more vulnerable in view of the high population depending on agriculture. Climate change, it is predicted will reduce yields by 4.5-9 percent, depending on the magnitude and distribution of global warming. (CIA, Factbook, 2014-15). People's livelihoods, especially in rural areas, are directly or indirectly affected by climate change. Climate change poses a direct and serious threat to the livelihoods of millions of people in India. They depend pre-dominantly on agriculture and natural resources (Kates et al. 2011).Negative impacts of climatic change are such as cereal productivity to decrease by 10-40 percent by 2100, greater loss expected in Rabi crops. Every 1°C increase in temperature will reduce wheat production by 4-5 million tones, and with increase in 2°C temperature the yield of rice and wheat will decrease i.e. 8.5 and 12.2 quintal per hectare, respectively. Further increase in melting of glaciers in Himalayas will affect availability of irrigation in the Indo- Gangetic plains (Aggarwal, 2008).In many regions throughout the world, temperatures and precipitation impact the production potential of major crops (Le 2016; Devkota et al. 2013; Yang et al. 2018). In rice production, modeling studies project country specific variations in rice production due to climate change in Bangladesh (Sarker et al. 2013), China (Bachelet et al. 1995), India (Mall and Agrawal 2002), Pakistan (Abid et al. 2015), and Nepal (Karn 2014; Adhikari et al. 2017). Reported that, rice yield declines by 5 to 7 percent with 1°C increase in mean day. Tamil Nadu is one of the economically well-equipped states in the nation with major core industries and agro based industries. It is also considered as agriculture predominant state with the adequate cultivatable lands. Cauvery delta place of Tamil Nadu is considered as "Nerkalanchiyam" (Land of Paddy cultivation). Rice is one of the most important staple food crops which are predominantly grown in the Cauvery river basin, which is also known as the rice bowl of Tamil Nadu (Geethalakshmi., et al.2011).The objectives of the present study are to know the attitude of paddy growers towards climate change. Very few studies have intensively examined attitude of farmers towards climate change on agriculture.

MATERIAL AND METHODS

The Cauvery Delta Zone (CDZ) of Tamil Nadu State formed the universe of the study since the zone was the major rice production environment which produces more than 40 per cent of the state rice production. The districts of Thanjavur, Thiruvarur and Nagapattinam were chosen purposively since these districts constitute around 70 per cent of the total ayacut area of Cauvery canal. CDZ as universe, districts as the first stage, taluks as second stage, blocks as third stage, villages as fourth stage and the ultimate sampling units were the farmers. In total 240 respondents were selected. The primary data was collected using well-structured and pre tested interview schedule from the respondents. The responses were measured using five-point continuum scale ranging from Strongly Agree', 'Agree', 'Undecided',' Disagree', and 'Strongly Disagree with scores of 5,4,3,2 and 1, respectively. The respondents were asked to choose their responses for each of the statement on a five-point continuum. With the total score obtained from the attitudinal statements/items, the respondents were classified into three categories such as low medium and high level of attitude towards climate change on agriculture. The mean score was worked out to present the statement wise attitude of respondents towards climate change.

RESULTS AND DISCUSSION

Attitude is an organized predisposition to think, feel and perceive and behave towards a cognitive object. Climate change directly affects agriculture production. Hence, it become necessary to find out the attitude of respondents towards climate change. Based on the data and minimum and maximum possible scores, the respondents were grouped into three categories low medium and high level of attitude towards climate change on agriculture. The frequency and percentage was calculated and presented in the tables 1-2.

Overall attitude of respondents towards climatechange

The existing overall attitude level of respondents towards climate change was studied and the findings are presented in Table 1. From the Table 1, it could be seen that more than half of the respondents (59.58 %) were found to possess high level of attitude towards climate change and nearly one -fourth of the respondents (32.50 %) had medium level of attitude and only (7.92 %) of the respondents had low level of attitude towards climate change. It could be concluded that majority of the respondents had high level of attitude towards climate change. In general, the age and farming experience of an individual would tend to develop a high level of attitude towards climate change. The higher the age would be higher the farming experience helps to understand the changing nature of climatic condition. This may be probable reason for majority of the respondents had high level of attitude towards climate change.

Statement wise attitude of respondents towards climate change

This section highlights the statement wise attitude of respondents towards climate change. The collected data were analyzed and presented in Table 2. The results pertaining to the statement wise attitude of respondents towards climate change are presented the above Table 2, it was found that the statement 'climate change has the serious problem and action to be taken on the livelihood of farmers' was received first rank with the mean score of (4.79 MS).The reason may be due to the increased in temperature, uneven rainfall and flooding and extreme weather impact the yield of paddy crops and their livelihood. The statement 'climate change affects the food security, income and employment of villagers' ranked second with the mean score of (4.76MS). The reason may be due to that climate change affects the yield of crops as well as livestock's which has direct impact on the income & employment of the villages. The statement 'rise in temperature, uneven rainfall, heat waves and other extreme weather due to effect of climate change' was ranked 3rdwith the mean score of (4.61MS), followed by the statements 'climate change causes lower production and productivity of crops',' due to climate change there is an increase in farm and home expenditure' and 'due to the climate change the incidence of crop pests and diseases is more' ranked 4^{th,} 5th and 6th respectively with the mean score of (4.48MS), (4.35MS) and (4.28MS). Unfavorable climatic condition reduces the crops yield & increases the pest and diseases incidence and weed proliferation which in turn, reduces the productivity.

The statement 'working capacity is affected due to climate change' was ranked seventh with the mean score of (4.23MS), followed by the statements' high cost is needed to adapt the climate resilient practices at farm level', 'climate change is one of the reasons responsible for reduction in forest area' was ranked 8th and 9th respectively with the mean score of (3.96MS) and (3.95MS). Climate change such as high temperature and heavy rainfall affect the working capacity of the farmers, hence most of the respondents had high level of attitude towards the statement working capacity is affected due to climate change. The statement 'adaptation strategies are the best way to increase the farmer's income in rice cultivation' was ranked 10th with the mean score of (3.89MS), followed by the statements' as a result of climate change the rate of migration from farming to urban areas for employment is increased',' climate change prevents the farmers participation in agricultural activities', 'human activity is responsible for climate change' and 'adaptation strategies against climate change are a high risk strategy' was ranked 11th , 12th, 13th and 14th respectively with the mean score of (3.82MS), 3.50MS), (3.25MS) and (2.07MS). The villagers are forced to migrate from rural to urban areas due to production loss & unemployment caused by the changing climatic condition.

CONCLUSION

From the above results it is understandable that, majority of the respondents (59.58%) had high level of attitude climate change. Even though high level favourable attitude is giving hope for the extension professionals for better implementation of climate change based programmes to the paddy farmers in delta region.

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Table: 1 Distribution of respondents according to their overall attitude towards climate change

S.No	Attitude category	Number	Per cent
1	Low	19	7.92
2	Medium	78	32.50
3	High	143	59.58
Total		240	100.00

Table 2.Distribution of respondents according to their statement wise attitude towards climate change

S. No	Statement	MWS	Rank
1	Human activity is responsible for climate change.	3.25	XIII
2	Climate change is one of the reasons responsible for reduction in forest area.	3.95	IX
3	Climate change has the serious problem and action to be taken on the livelihood of farmers	4.79	I
4	Rise in temperature, uneven rainfall, heat waves and other extreme weather due to effect of climate change	4.61	III
5	Climate change affects the food security, income and employment of villagers	4.76	II
6	Climate change causes lower the production and productivity of crops	4.48	IV
7	As a result of climate change the rate of migration from farming to urban areas for employment is increased	3.82	XI
8	Climate change prevents farmers participation in agricultural activities	3.50	XII
9	Due to the climate change the incidence of crop pests and diseases is more	4.28	VI
10	Adaptation strategies are the best way to increase the farmer's income in rice cultivation	3.89	X
11	Adaptation strategies against climate change is a high risk strategy	2.07	XIV
12	High cost is needed to adapt the climate resilient practices at farm level	3.96	VIII
13	Due to climate change there is an increase in farm and home expenditure	4.35	V
14	Working capacity is affected due to climate change.	4.23	VII

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RESEARCH ARTICLE

Vincamine Efficacy on Scavenging Reactive Oxygen Species under *In vitro* Condition

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ABSTRACT

Reactive oxygen species could contribute to several human diseases if they are excessively generated in the body. The compound that possesses significant free radical scavenging ability are considered as potent antioxidant. Medicinal plants contain several bioactive constituents with significant free radical scavenging efficacies. The present study has examined the in vitro free radical scavenging (antioxidant) potential of vincamine using specific and standard colorimetric methods. Vincamine effectively scavenged DPPH radicals, hydroxyl radical, ABTS radicals, superoxide radical hydrogen peroxide and nitric oxide radical in a dose dependant manner and the antioxidant effect was comparatively lesser than that of ascorbic acid, the standard reference drug. The *in vitro* antioxidant ability of vincamine is thus explored in the present study using specific free radical scavenging assay system.

Keywords: Vincamine, free radicals, Superoxide, Hydroxyl radical, DPPH, ABTS.

INTRODUCTION

Reactive oxygen species (ROS), the most important and significant radical derivatives of oxygen, play a key role not only in the pathological conditions but also in the physiological processes. ROS such as superoxide and hydroxyl radical play a normal prominent role in killing pathogenic bacterial cells, toning smooth muscles and regulating the functions of blood vessels and internal organs [1]. Reactive oxygen species are highly harmful if they are overproduced in the system and could lead to several pathological diseases [2]. Proteins, lipids and nucleic acids are the major target of excessively generated free radicals, which can cause cell damage and defect in cell function [3]. Abnormal lipid peroxidation has been attributed to the pathogenesis of carcinogenesis. [4]. Altered status of lipid peroxidation has been shown in various pathological conditions [5, 6]. Humans are endowed with a defense mechanism against reactive oxygen species are termed as antioxidant. Two types of antioxidant systems, namely enzymatic and non-enzymatic antioxidants, fight against reactive oxygen species and act synergistically to scavenge the free radicals. A defect in antioxidant defense mechanisms has been shown in several pathological conditions [6, 7]. The enzymatic antioxidants are lipophilic antioxidants, which include superoxide dismutase, glutathione peroxidase and catalase. The non-enzymatic antioxidants are water soluble antioxidants, which include vitamin C, vitamin E and reduced glutathione. Vincamine, a naturally occurring monoterpenoid indole alkaloid, is present in the plant Vinca minor.Vinca minor has around25-65% of vincamine , an indole alkaloids. Vincamine has been recommended to use as a dietary supplement in the United States. It has been used as a peripheral vasodilator as well to combat the aging process of humans. Fayed et al., [8] reported that vincamine has the ability to reduce the iron content of brain, which could be due to its inhibitory effect on iron mediated oxidative stress. Rasheed et al., [9] recently reported the anti proliferativepotential of vincamine in Alveolar basal epithelial cell line.Nandini et al., [10] reported the antidiabetic, and antioxidant efficacies of vincamine in streptozotocin induced diabetic rats. Salam et al., [11] explored the neuroprotective and antioxidant potential of vincamine at low therapeutic doses in the rats. A spectrum of pharmacological effects of vincamine including nootropic and vasodialating properties has also been reported. [12, 13]. It has been reported that Vincamine has the ability to alleviate amyloid beta 25-35 peptide induced cytotoxicity in PC 12 cells [14]. The present study revealed the in vitro free radical scavenging potential of vincamine using several *in vitro* antioxidant colorimetric assays.

MATERIALS AND METHODS

Chemicals

Vincamine was purchased from Sigma Aldrich, Bangalore, India. All other chemicals used were purchased from Hi Media Laboratories Pvt, Ltd. India.

Free radical scavenging assay

The free radical scavenging activity of vincamine was examined using the spectrum of specific free radical scavenging assays and the results obtained were compared with reference drug ascorbic acid. The entire free radical scavenging test was done in triplicate and the results are presented as mean±SD and a graph was prepared using concentration against % of inhibition of radicals.

DPPH radical scavenging assay

The DPPH radical scavenging effect of vincamine was assed according to the procedure of Blois [15]. A known concentration of vincamine and the reference drug ascorbic acid (20, 40, 60, 80 and 100 μg/ml) was added to the methanolic solution of DPPH. This mixture was kept in dark for half an hour and then the absorbance of the solution was read at 517nm.

The DPPH radical scavenging potential of the vincamine and ascorbic acid was calculated using the formula.

$$\% \text{ of scavenging effect} = \frac{\text{Absorbance of the control} - \text{Absorbance of the test}}{\text{Absorbance of the control}} \times 100$$

The free radical scavenging activity was expressed in terms of $IC_{50,}$ which represents the concentration of vincamine / ascorbic acid that can able to scavenge 50% of free radicals.

ABTS scavenging assay

ABTS radical scavenging activity of the vincamineand ascorbic acid was estimated by the method of Miller et al., [16]. This method measures the efficacy of the test compound to scavenge the ABTS radical in the aqueous phase, which was measured at 734nm.

Superoxide radical scavenging assay

The effect of vincamine on scavenging the superoxide radical was measured using the procedure of Nishimiki et al., [17]. This method determines the ability of the superoxide radicals that are generated from PMS/NADH system to reducenitroblue tetrazolium to a purple formazan, which was measured at 560nm.

Hydroxyl radical scavenging assay

The hydroxyl radical scavenging potential of vincamine was determined as per themethod of Halliwell et al., [18]. This method measures the ability of hydroxyl radicals to degrade deoxyribose and the products generated reacts with TBA formed a pink colour chromogen, was read at 532nm.

Nitric oxide radical scavenging assay

Garrat et al., [19] procedure was used to determine the nitric oxide radical scavenging effect of vincamine. This method is based on the reaction of nitric oxide, generated from sodium nitropruside with oxygen and the generated nitrates and nitrites on reaction with Griess reagent formed a chromphore, which was read at 546nm.

Hydrogen peroxide scavenging assay

The method of Jayaprakasha et al., [20] was utilized to assess the hydrogen peroxide scavenging effect of vincamine. This method is based on the measurement of the disappearance of H_2O_2 at a wavelength of 230nm.

Reducing power assay

Reducing powder of the vincamine was assayed using the method of Oyaizu et al., [21]. This method determines the effect of vincamine to reduce potassium ferricyanide to ferrocyanide, which on further treatment with ferric chloride to form a ferric-ferrous complex. The absorbance of the complex was read at 700nm.

STATISTICAL ANALYSIS

Values are expressed as mean ± SD. The IC50 concentration of the test compound was measured using graphical calculation method. The statistical significance between the groups is analyzed using one way analysis of variance followed by Duncan's multiple range test. The p value less than 0.05 is considered statistically significant.

RESULTS AND DISCUSSION

The present study evaluated the *in vitro* free radical scavenging effect of vincamine using specific colorimetric assays. The free radical scavenging potential of vincamine was compared with the reference compound ascorbic acid. Figures 1-7 show the free radical scavenging effect of Vincamine as compared to ascorbic acid on DPPH radicals (IC_{50} 74.2 μg/ml for vincamine and 46.8 μg/ml for ascorbic acid), ABTS (IC_{50} 28.3 μg/ml for vincamine and 22.2 μg/ml for ascorbic acid), $O_2^{\cdot-}$(IC_{50} 64.7 μg/ml for vincamine and 56.1 μg/ml for ascorbic acid), hydroxyl radicals (46.2 μg/ml for vincamine and 34.7 μg/ml for ascorbic acid), $NO^{\cdot}$(IC_{50} 58.2 μg/ml for vincamine and 45.4 μg/ml for ascorbic acid), H_2O_2 (IC_{50} 52.8 μg /ml for vincamine and 38.2 μg/ml for ascorbic acid) and reducing power (increasing with increasing concentration) respectively. Vincamine significantly inhibited various free radicals and hydrogen peroxide in a dose dependent manner and the scavenging potential was comparatively lesser than that of ascorbic acid. *In vitro* free radical scavenging assays could play a pivotal role in the identification of antioxidant or free radical

scavenging potential of several medicinal plants and their bioactive constituents. In recent years, the antioxidant potential of natural products have been explored worldwide using these *in vitro* free radical scavenging assay system. The present study explores the *in vitro* free radical scavenging potential of vincamine using a spectrum of free radical scavenging assays. In recent years, researchers across the world prefers DPPH assay to evaluate and confirm the antioxidant potential of natural products under *in vitro* condition [22]. The antioxidant potential of the test compound was analysed by their ability to reduce the violet colour solution of DPPH into yellow coloured product, diphenylpicryl hydrazine. In the present study, vincamine effectively scavenged the DPPH radicals, which explores its antioxidant efficacy. This antioxidant effect could be due to its ability to transfer a hydrogen atom or electron to neutralize the effect of DPPH radicals. ABTS radical scavenging assay is commonly employed to assess the total antioxidant capacity of the test compound. This method could provide information about the relative ability of the test compound to scavenge the ABTS radicals, which are produced by the reaction of potassium persulfate with the ABTS salt. The present study noticed a considerable and appreciable antioxidant activity of vincamine with ABTS radicals. Superoxide radicals are dangerous free radicals as they can be converted into potent hydroxyl radicals in the biological systems, which can able to cause extensive damage to the DNA and other cellular components [23]. The superoxide radical scavenging ability of the test compound relies on the conversion of NBT into purple coloured NBT diformazan by the superoxide radicals. The present study noticed that vincamine has the good ability to scavenge the superoxide radicals that are generated using PMS/NADH system. Hydroxyl radicals are deleterious reactive oxygen species, which can able to reduce disulfide bonds in the protein structure and can interact with cell membrane lipids to initiate a free radical mediated chain reaction known as lipid peroxidation. Abnormal status of membrane lipid and protein oxidation could result in altered membrane fluidity and permeability [24, 25].

The present study observed that vincamine effectively scavenged the hydroxyl radicals under *in vitro* conditions and thus has the ability to protect the cellular membrane during oxidative stress. Nitric oxide has been regarded as one of the major oxides of nitrogen and is a free radical and a weak oxidant. Nitric oxide can able to regulate several biological functions and could serve as a principal physiological regulator through reversible interactions with the heme proteins. Nitric oxide could play a vital role in increasing blood flow and lowering blood pressure [24, 25]. Excessively generated nitric oxide causes brain damage and may thus lead to various neurodegenerative disease [25]. In the present study, nitric oxide radicals are scavenged in a considerable manner by vincamine under *in vitro* conditions. Hydrogen peroxides (non-reactive oxygen species) are formed during normal cellular metabolism. Over production of H_2O_2 could result in oxidative stress as H_2O_2 are converted to potent hydroxyl radicals, which could cause extensive damage to cellular components and thus causing several diseases. The present study noticed that vincamine has the ability to scavenge H_2O_2 as well under *in vitro* conditions and thus can protect ROS mediated oxidative damage. Reducing power assay method could help to find out the reduction potential of the test compound and is depend on the formation of ferric-ferrous complex. Reducing powder assay is generally considered as the most convenient method as well as utilized to rapidly screen the antioxidant potential of the test compound. In the present study vincamine significantly exhibited the reducing power and thus considered as a good free radical scavenger.

CONCLUSION

The present study thus highlighted the *in vitro* free radicals scavenging potential of vincamine using various specific *in vitro* free radical scavenging assays. The present study noticed that the antioxidant activity of vincamine is dose dependant and the effect was however lesser than that of ascorbic acid. Though the effect of vincamine is lesser that that of ascorbic acid, it exerted a similar effect to that of ascorbic acid at one and half to double the concentration of IC_{50} values of ascorbic acid.

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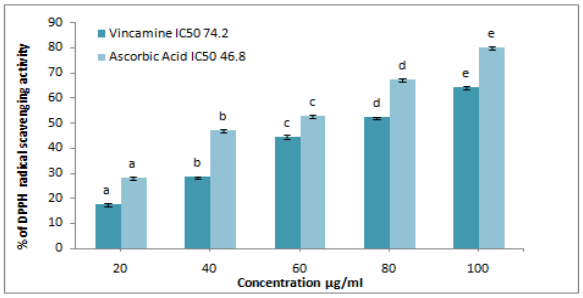

Figure 1: DPPH radical scavenging potential of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

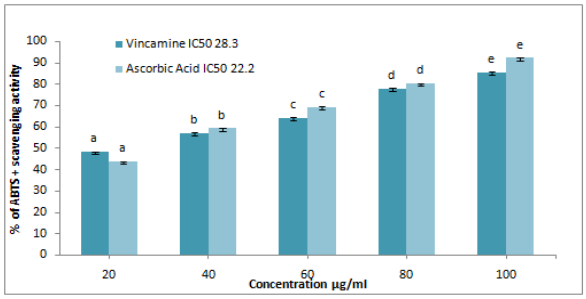

Figure 2: ABTS radical scavenging potential of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

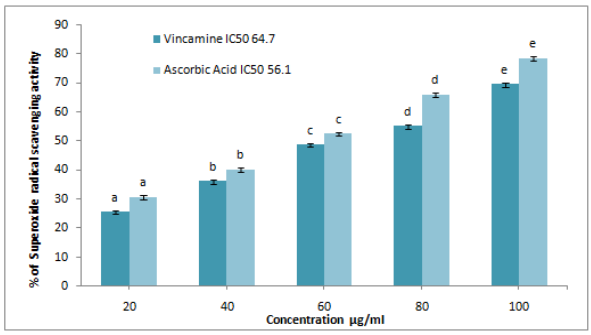

Figure 3: Superoxide radical scavenging potential of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

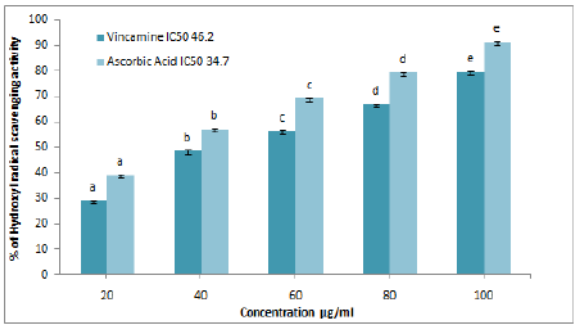

Figure 4: Hydroxyl radical scavenging potential of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

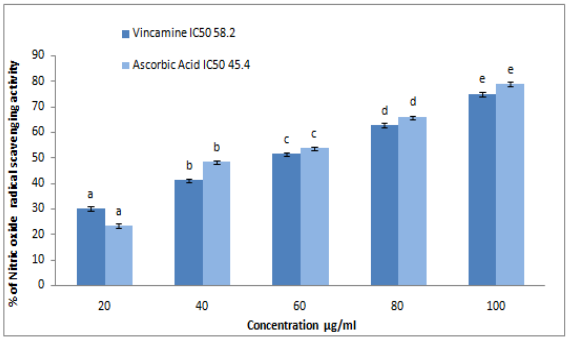

Figure 5: Nitric oxide radical scavenging potential of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

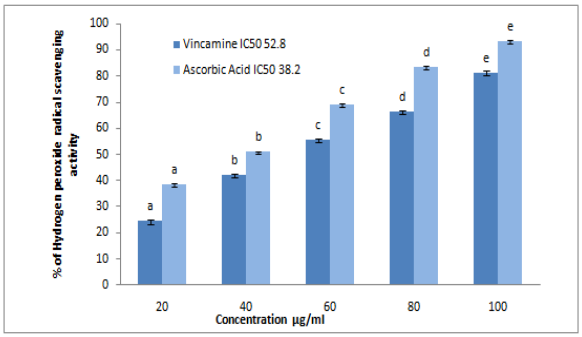

Figure 6: Hydrogen peroxide scavenging potential of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

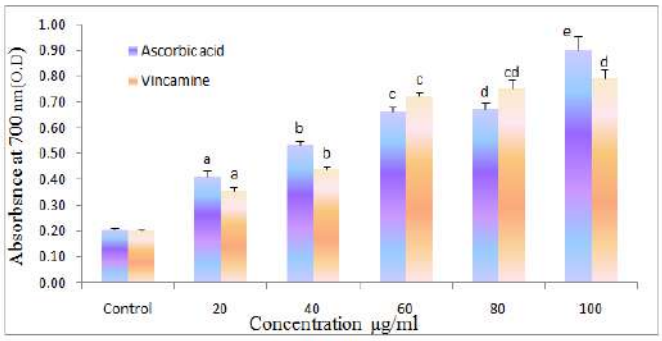

Figure 7: Reducing power efficacy of vincamine. Values (mean±SD; n=3) with different superscript differ significantly at p<0.05

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RESEARCH ARTICLE

Tongue Print Biometric System using Generative Adversarial Networks

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ABSTRACT

Biometric technology has received a lot of attention in recent years. One of the emerging biometric traits is the tongue print. This research aims to create an autonomous deep learning framework for tongue print authentication systems with a high recognition rate and rapid authentication. A hybrid model is proposed combined VGG16 & GAN. The combined architecture which attains a Precision of 99.1% & Recall of 99.2% and based on the F1-Score, the model's total performance is 99.4%. The designed method can improve the recognition rate and the recognition speed for tongue print biometric systems.
Keywords: Biometrics, Tongue print, Authentication, Identification

Keywords: VGG16 & GAN, Authentication, combined architecture, biometric traits.

INTRODUCTION

In today's digitally connected world, automated identification systems are crucial for security and privacy [1]. "Bio" signifies life and "Metrics" means to measure [2]. Biometrics measures human features to verify an individual's identification. Biometrics System automates individual recognition. Biometrics are employed in information systems to manage identify and limit access. It also identifies people in undercover organisations. Depending on the circumstances, identification might be confirmed or rejected [1]. Authentication, or verification, is at issue. Authentication verifies a user's claimed credentials against their real ones. Authentication includes one-to-one matching with the database or IDs. "Yes" implies the person is the stated identity; "No" means the claim cannot be confirmed. Authentication is used for logical or physical access. Identification is the challenge of determining a subject's identify from a collection of known identities (closed identification problem) or otherwise (open

identification system) [1]. Identification searches the individual's templates against database templates to identify them. Database search for identification. The identification search yields a group of candidate matches with the greatest similarities or exceeding the preset threshold. Physical entry or law enforcement often need identification.
Humans prefer to use passwords or PINs for authentication and identification. Theft, sharing, or guessing may compromise passwords. Thus, passwords should be alphanumeric, restricted in duration, distinct in each programme, and often updated. Policies make passwords hard to remember. Some scribble passwords on keyboards or displays. Humans utilise "what they have," such as identifying objects, to verify identity. Identification cards, smart cards, and signature stamps are examples. Unauthorized people may take IDs and use them illegally. To solve these flaws, biometric, "what you are," is presented. Biometric technologies can be compared using parameters such as universality, uniqueness, permanence, collectability, performance, acceptability and circumvention.

Universality
Every individual using the biometric system should have the same biometric characteristics.

Uniqueness
The biometric features must be distinctive from one individual to another in the biometric system.

Permanence
The biometric features should be resistance to aging. It should not change much across a period of time.

Collectability
The biometric features should be easy to collect, using acquisition devices or sensors, and measurable.

Performance
How the biometric system performs in term of accuracy, speed and robustness.

Acceptability
How the public or private users receive the biometric technology and willingness of using the biometric system.

Circumvention
How easy the biometric features being imitated, substituted or mimicked. For example, fake finger in fingerprint identification.

Why Tongue Print Authentication System?
Because of the growing demand for new methods of identifying people, some researchers have looked into the uniqueness of the tongue. Their preliminary findings suggest that it could be as unique as fingerprints, and that tongue prints vary even in identical twins [4]. This is in response to the growing need for new methods of identifying people. Different tongues have distinct differences in the size, contour, and feel of their surfaces. It is the sole organ that may be found inside the mouth of a human being (keeps safe). We don't normally perform any exterior work while we do this, with the exception of pushing our tongue out. Other means of identification used for security, such as a person's eyes or fingerprints, are more likely to be common and open to scrutiny. While our tongue is well-formed and safe in terms of data identification, the Tongue Id Recognition system cannot be used without the express authorization of the user. No one will be able to quickly take out your tongue and scan it in a regular manner even if you are sleeping or occupied with anything else. Because our tongue is kept within our mouth at all times, it is impossible to leave any trace of tongue id recognition on anything, not even by accident. Unlike iris scans and fingerprints, which may be readily replicated, this trait cannot be easily duplicated or imitated [5, 6].

Description of Tongue
The tongue is a muscle organ located in the mouth of most animals and mammals. Its primary function is to manipulate food prior to it being chewed by the teeth and swallowed. mostly because it is the most important part of

digesting food and tasting it. The tongue acts as a taste checker and is covered with a large number of taste buds that are known as the "Lingual Papillae." It has a high degree of responsiveness and sensitivity, similar to that of other nerves and organs, and it is maintained moist by the fluid known as "Saliva" (supplied by nerves and blood vessels). Speech production is the most significant function of the tongue in humans, but other species use their tongues for vocalisation as well. The front, or oral, section of our tongue is different from the pharyngeal, or rear, half, which is located in the middle of the tongue. The tongue is a muscle organ located in the mouth of most animals and mammals. Its primary function is to manipulate food prior to it being chewed by the teeth and swallowed. mostly because it is the most important part of digesting food and tasting it. The tongue acts as a taste checker and is covered with a large number of taste buds that are known as the "Lingual Papillae." It has a high degree of responsiveness and sensitivity, similar to that of other nerves and organs, and it is maintained moist by the fluid known as "Saliva" (supplied by nerves and blood vessels). Speech production is the most significant function of the tongue in humans, but other species use their tongues for vocalisation as well. The front, or oral, section of our tongue is different from the pharyngeal, or rear, half, which is located in the middle of the tongue. The top surface of the tongue, known as the dorsum, is split into two symmetrical halves by a groove known as the 'Median Sulcus,' which runs along the middle of the tongue. The foramen cecum denotes the conclusion of the division that is about 2.5 centimetres in length, measuring from the base of the tongue to the opening of the terminal sulcus. During the process of embryonic development, the formation of the thyroglossal duct takes place during the descent of the thyroid diverticulum. The foramen cecum serves as the site of attachment for this duct.

LITERATURE REVIEW

Biometric ID systems work similarly. First, biometrics are captured. After digitising the sample, they adjust it and create a data set or extract features. The last stage compares the input sample to known samples in the database (a one-to-many comparison) and provides a similarity score. The input sample may also be compared with one other sample (a one-to-one comparison) to determine whether they are the same [9]. Biometrics technology, a component of technological innovation [10,11], has gained importance in recent years, especially in machine learning and AI. This quest led to deep learning [12-14]. Deep learning image classification requires convolutional structures. To train a deep network, start with a lot of labelled data. [15,16]. This may be done using a pre-trained algorithm like SqueezeNet, VGG, Inception, or ResNet, or ImagNet. In 2007, Liu et al. created the first tongue print recognition system [17]. Bade et al. [18] proposed 2D dual-tree complex wavelet transform tongue recognition algorithms. Li Qet al. [19] and Manoj Diwakar et al. [20] studied tongue print pictures as a biometric characteristic. Humans were identified using tongue print histograms. They also created a tongue database. The tongue's cross-section may be used to differentiate between identical twins, indicating that it can be used as a biometric to identify a person. Radhika et al. [22] observed that tongue prints beat other biometrics. Bob Zhang and Han Zhang [23] used geometric tongue print features to diagnose patients. Stefanescu et al. [24] classify languages by morphology. Salim Lahmiri [25] used wavelet transformation to check six statistical characteristics from a tongue print. Ryszard S. Choras [26] proposed steerable filters and the Weber Law Descriptor. Zhang et al.[27] used form and texture.Their work explores geometric factors for form characterization and textural codes as textural characteristics. Sivakumar et al. [28] extracted tongue textural patterns using LBP. Personal identity features are learned using a linear SVM.

CNN extracts and compares training picture characteristics effectively. CNNs are taught via back propagation, random gradient descent, and micro batch learning [29]. Deep learning architectures are used to fix computer-perception errors. ResNet [30] adds an identity component to the CNN architecture, allowing for increased CNN depth while reducing learning degradation. MobileNet [31] uses reversed residuals to construct residual connections between bottleneck layers. The Shuffle-Net [32] is tiny and portable. CNN is wireless-equipped. Low computational power makes it ideal for many applications. Low computational cost is due to channel shuffling and point-wise grouping convolution. Efficient-Net [33] presented new CNN performance techniques. Efficient-Net believes a CNN's effectiveness may be increased by adding density, layers, breadth, and filters. These ways may improve a biometric security system's performance. None of the published tongue print identification systems are ideal; each

has limitations introduced by computer vision and machine learning. Deep learning approaches on tongue print photos still need development.

METHODOLOGY

A generalized block diagram of the tongue print authentication system is presented in Figure 2. In order to extract features, the CNN architectures which are pretrained on the ImageNet dataset [83] are used in this work. For feature extraction and training, MATLAB 2021b and WEKA tool are used. The Weka tool version (3.6.9) is used to train and test using the extracted features, and to classify the obtained deep features SVM & SOFTMAX Classifiers are used. The proposed work is conducted on Intel(R) Core (TM) i7-6700 CPU at3.40 GHz with 8 GB memory. All the WEKA methods are used with their default parameters. The dataset is divided randomly in the ratio of 80% training and 20% testing ratio. The training and the testing images are fed into the proposed models and the features are extracted. At last the deep learning models will identify the tongue print images.

Database

In tongue print identification is still in its initial stages and requires further study and planning to deploy. The establishment of a database is essential for the purpose of identification; however, there is not yet a standard database available. To achieve the objectives of the study, especially as it employs deep learning, in this work the database captured from the 50 students both male & female students from the age group (18 – 30 Years) of Vinayaka Mission's Kirupananda Variyar Engineering College, Salem, Tamil Nadu, India. The dorsal tongue is photographed using a smartphone camera under uniform lighting settings. Four distinct images of the dorsal surface of the tongue are acquired for each person, each with a unique orientation, size, and shape. As a result, our database has 200 images. The sample tongue images are shown in figure 3.

Performance Evaluation Metrics

To evaluate the performances of the implemented model different performance measure metrics such as accuracy, precision, recall, specificity, and F1-score are used. To evaluate these metrics, several indices such as True Positive (TP), False Positive (FP), True Negative (TN), and False Negative (FN) are the four labels derived from the confusion matrix is required as shown in figure 4. TP is the correctly classified images in a particular category. FP is the number of wrongly classified images, whereas TN is the sum of the correctly classified images in all other categories and FN is the number of misclassified images from the relevant category.

Precision

The precision is calculated as the ratio between the number of Positive samples correctly classified to the total number of samples classified as Positive (either correctly or incorrectly). The precision measures the model's accuracy in classifying a sample as positive.

$$Precision = \frac{TP}{TP+FP} \quad (3.1)$$

Recall

The recall is calculated as the ratio between the number of Positive samples correctly classified as Positive to the total number of Positive samples. The recall measures the model's ability to detect Positive samples. The higher the recall, the more positive samples detected.

$$Recall = \frac{TP}{TP+FN} \quad (3.2)$$

F1 Score

The F1 score is defined as the harmonic mean of precision and recall. The goal of the F1 score is to combine the precision and recall metrics into a single metric. At the same time, the F1 score has been designed to work well on imbalanced data.

$$F1 = 2\frac{Presision*Recall}{Presision+Recall} \quad (3.3)$$

Generative Adversarial Networks

One of the most significant developments that has been made in recent years in the field of artificial intelligence is known as deep learning. It makes it possible to find computational solutions to situations that are difficult to define using a mathematical model or an algorithm that is predictable. Additionally, it makes it possible to apply automated solutions to situations that have a fundamentally subjective nature. Generative modelling is an unsupervised learning task in the field of machine learning that involves automatically discovering and learning the regularities or patterns in input data in such a way that the model can be used to generate or output new examples that plausibly could have been drawn from the original dataset. The goal of this type of modelling is to make it possible for the model to generate or output new examples that plausibly could have been drawn from the dataset. GANs will propose an attention mechanism in this chapter that can dynamically bring significant tongue print characteristics to the forefront of attention. This is done with the purpose of enhancing the model's capability of acquiring biometric characteristics for the purpose of identification. First, the VGG16 CNN, which has been pre-trained and performs better than other CNNs when given the job of authenticating a tongue print, is utilised as a visual feature extractor. This results in a tongue picture being encoded as CNN features (i.e., a tensor of feature maps at a given output of a chosen convolutional layer). These CNN features may be thought of as a new, more compact picture of activations, and they have the same number of channels as the filters that were employed in the convolution layer. After that, this new picture is divided into sub-parts of the same size so that local activations may be obtained in a variety of places that span the whole of the image. After that, these newly discovered local CNN features may be put to use to construct a sequence, which can then be fed into GAN and utilised to enable an attention mechanism that can detect significant portions or components in the CNN features. It does this by extending the effective pixel neighbourhood in each subpart and by optimising the information gain across several subparts of the CNN's features. In conclusion, prediction error is kept to a minimum all throughout the process of optimization.

Generative Adversarial Network (GAN)

The GoodFellow et al. [35] research group has only lately invented a new type of artificial neural networks called GAN. The model is an amalgamation of two neural networks, each of which is trained in an adversarial environment. The first network is made up of a generator, which is responsible for producing new data instances after receiving random noise as input. The input for the second neural network, which we will refer to as the discriminator, comes from both the generator and the data that was used for initial training. Following this, the discriminator examines each data instance in order to determine whether or not the data is genuine (that is, it originates from an actual training dataset) or whether or not it originates from the generator, as seen in figure 5. In principle, there will come a time when the generator will have reached the point where it can fully capture the training data distribution. As a result, the discriminator won't be able to determine whether or not the inputs come from the generator. After reaching this stage, the GAN is considered to have completed all of its training. In other words, the generator (*G*) takes in a vector of random noise *z* and generates a sample $Xfake = G(z)$. The input to the discriminator network *D* is real data and samples produced by the generator, and it outputs a probability distribution, which represent the probability of the data possible sources. Equation (5.1) summarizes the entire GAN training concept. The discriminator *D* is trained to maximize the log-likelihood to assigns correct label, while the generator (*G*) is trained to maximize the probability of *D* making a mistake (second term in the equation).

$$L = E\,[logP(Y = real|Xreal)] + E[1 - logP(Y = fake|Xfake)] \quad (5.1)$$

GANs are known to be unstable, and difficult to train. This leads the generator in many instances to produce poor samples. Therefore, several research papers directed their efforts in improving the stability of training.

RESULTS AND DISCUSSION

In this chapter the hybrid VGG16 + GAN is implemented in order to compare and quantify the performance with the proposed model for the efficient tongue print authentication. To evaluate the performances of the implemented model, the different performance metrics such as accuracy, precision, recall, and f1-score are used. The performances of the combined VGG16 & GAN are applied on the database. The experimental findings of the suggested frameworks are presented and discussed. Based on Table 1 it is observed that VGG16 & GAN achieves a Precision of 99.1% & Recall of 99.2% and based on the F1-Score, the model's total performance is 99.4%.

CONCLUSION

In recent years, biometrics has gained popularity. Tongue print is a developing biometric. Tongue prints will change identifying procedures since they can't be duplicated or applied without the person's consent/consciousness. This study uses a standard database of 50 male and female students from Vinayaka Mission's Kirupananda Variyar Engineering College in Salem, Tamil Nadu, India.A hybrid model is proposed combined VGG16 & GAN. The combined architecture which attains a Precision of 99.1% & Recall of 99.2% and based on the F1-Score, the model's total performance is 99.4%. The designed method can improve the recognition rate and the recognition speed for tongue print biometric systems.

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Table 1. Results of VGG16 and GAN

Precision	Recall	F1 Score
0.991	0.992	0.994

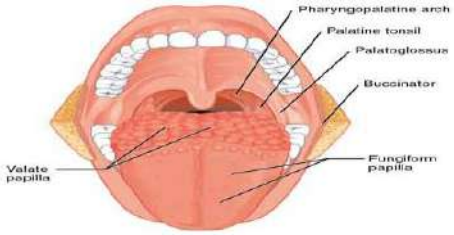

Figure 1. Structure & Surface of the Tongue

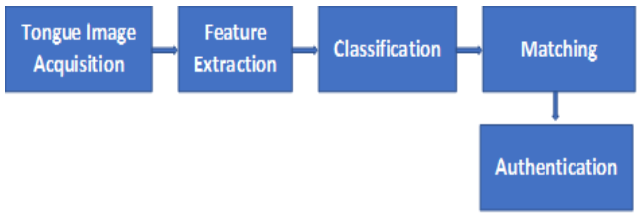

Figure 2. Block diagram of tongue print authentication system.

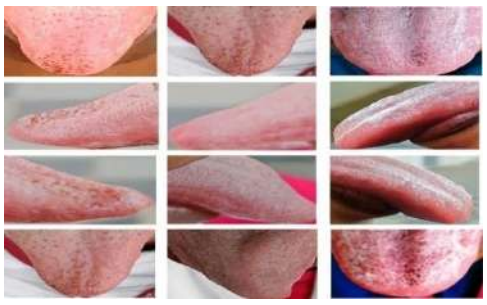

Figure 3. Sample images from the database.

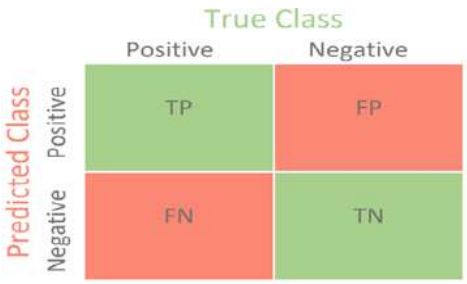

Figure 4. Confusion Matrix

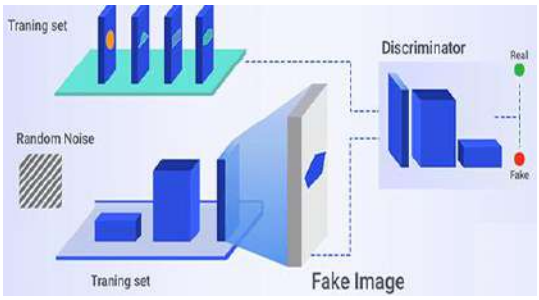

Figure 5. GAN Architecture

RESEARCH ARTICLE

Deteriotion of Blood Stain on Crime Scene; A Threat to Evidence

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ABSTRACT

Microorganisms are attained nearly everywhere on earthy surface along with remarkably diversity and ubiquitous in communities. Microbial forensic help to determine the involvement and analysis of evidences of criminal cases mainly based on forensic attribution, ranging from bioterrorism, bio-crime, frauds, 987`utbreaks and transmission of pathogens/ accidental release of a biological agent/toxin. In these biological materials of human which are rich source of proteins, carbohydrates, lipids, trace elements as well as water, provide a virtuous milieu for the growth of microbes. Degradation of these biological materials raise a challenge in the downstream processes of forensic DNA typing technique, such as short tandem repeats (STR) DNA typing. Microbial degradation yields improper or no PCR amplification, heterozygous peak imbalance, DNA contamination from non-human sources, degradation of DNA by microbial byproductsetc. Conventional methods rely on the detection of antigens or enzymatic activity by limiting the detection, sensitivity and specificity of old forensic samples. It has been described earlier thata qPCR assay is effective in age estimation of bloodstains stored in an environmentally controlled laboratory for periods of up to one year. In this study, the effect of the environmental conditions on the rate of RNA degradation during storage was analysed.

Keywords: Blood sample. DNA, biological samples, degradation, environmental etc.

INTRODUCTION

During forensic investigation, several types of evidences are attained in distinct forms from scene of occurrence. These evidences lead the investigation to establish the link between suspect, victim and crime scene[1]. Blood is the most common form of evidence which is encountered from the crime scene. Since, it encompassed the valuable information as DNA, it has become milestone of forensic tools of investigation. It is found on the clothing's, bedding, curtains, upholstery and carpets, which may carry stains either due to wiping or spattering by the perpetrator. Thorough analysis of bloodstain patterns can provide critical facts about the crime, what has occurred during the expedition of violent crime which resulted with different shapes of bloodstain patterns[2].Blood can be recuperate in various forms from the crime scene such as in form of blood pool, smears, semi-liquid from and dry blood flakes.

Properties of Blood

Blood is a fluid connective tissue aids in the transportation of oxygen and prime nutrients to all the cells and brings carbon dioxide and other wastes. Blood is composed of 45% of cellular part and 55% of plasma. It is slightly alkaline in nature having a pH ranging from approximately 7.35-7.45 and 38°C of temperature[3].Plasma carries proteins such as fibrinogen or thrombin and traces of insulin, bilirubin etc. It contains some clotting factors such as thrombocytes, glucose and dissolved ions. Blood also helps in the regulation of temperature of human body. Possess a defensive role against certain microbes and pathogens. It comes in the category of tissue as it has a composition of cells, also fluid in nature as having 90% of water in the plasma[4]. It has no shape and dimensions of its own due to its fluidity as like other liquids. It casts and adjust the shape of its surroundings on numerous surfaces from discrete impact angles thus forming the different shapes of bloodstains on the point of impact such as wall, floor, furniture etc. For interpreting the morphology of blood cast all through the crime scene, the primary knowledge concerning its physical characteristics such as specific weight, relative density, viscosity, surface tension, adhesion, cohesion and capillarity is necessary.

Determination of shape and size of blood drops from crime scene

The size and shape of a bloodstain depends on the height and velocity of a blood drop. One drop of blood can adhere on the smooth surface from a perpendicular height having a rounded shape with pointed edges. If the angle of impact is not perpendicular subsequently the shape of drop will be somewhat ellipsoidal. Velocity has a significant role in the angle formation of drops. Angle of impact plays a critical role in the formation of bloodstain[5]. If the surface is not regular or smooth, it can interrupt the surface tension or cohesive forces of the droplet and this may lead to the disintegration of the droplet. Hence the resulting bloodstain cannot give the precise information regarding the impact angle. If the height is less from the surface, the drop appears big is size and if height is more then drops appear small in size[6].It is quite difficult to calculate the angle of impact when the surface is textile of some kind, especially made up of natural fibres as they absorb the blood. In such cases time of occurrence of crime committed can be interpreted by measuring the clotting time and capillary action of blood. At the crime scene, some secondary or satellite blood stains may be recovered which gives the additional information such as the position of the perpetrator, location of actual crime scene etc[7].

By analysing the morphological aspects the blood stains a lot of valuable information is obtained, such as:

- By measuring the ellipsoid shape of a dried blood drop the angle it was cast from can be determined.
- By calculating the clotting time and capillary action of blood, the approximation can be made about the time crime was committed.
- By taking the measurement of the diameter of blood droplet and considering the morphological characteristics depending on the properties of the surface it fell on, the height from which the blood was cast from can be determined.

Microorganisms decaying blood evidences

Evidences collection, preservation and packaging play a significant role and forensic investigator must follow preventive measures at crime scene. Any kind of delay in examination or improper preservation lead towards

destruction of evidences. In such cases, by studying the life span/ time duration of a particular microbe, one can estimate the results. The growths of microbes depend on the various factors such as optimum temperature, pH and time[8]. In a study, it has been studied that the growth of certain microbes on the blood evidence causes the deterioration of blood evidence. The growth of certain microbes (such as gram positive and gram-negative bacteria, fungi etc.) over blood evidence is seen in recent studies. They grow at a range of temperature and pH thus causes the decaying of the blood evidence. It has been studied that over a span of 1-4 weeks at temperature (4^0, 25^0, 37^0), the manifestation of microbial growth spotted in terms of smell, colour, and humidity and coagulation status[9]. Essentially, the breeding plaques were scrutinized macroscopically with naked eye, magnifying glass and stereo microscope used for evaluating the colour, odour, colonization type and haemolytic status. During macroscopic examination, the samples were confiscated from the colonies were painted with simple or complex staining methods. In Microscopic examination bacteria and fungi were observed[10]. At the end of microscopically examination of samples, species identification of microbes was performed by exploiting API 20C and 20E, biochemical methods and commercial identification kits. Since humidity has a prominent role in the growth of the microbes, that's why, the blood samples are always dried first at room temperature then, preserved[11]. Some of the grown microbes (bacteria and fungi) includes; *Aeromonas sp., Aspergillus sp., Bacillus sp., Bacillus subtilis*, *Corynebacterium sp. Escherichia coli, Micrococcus sp., Micrococcus luteus, Mucor sp., Penicillium notatum, Penicillium sp., Koagulase Negative cocci, Sarcina sp., Staphylococcus aureus Streptobacillus sp., Streptococcus agalactiae , Streptococcus sp. Etc.* Tab. Potent micro-organisms present on the forensic case exhibits to stimulate the degradation. By studying the life span/optimum conditions of growth of these microbes in blood stain can help to estimate the time when the crime has been committed or when the samples are collected. This can help to resolve such cases in which samples are damaged due to microbial action.

Morphological changes of Blood spatter

Analysis of bloodstain patterns in the field of forensics having a great significance to analyse the blood traces found at the crime scene. Following a bloodshed event, there are certain possibilities of recovering a blood pool from the scene of crime. In some cases, dry blood stains, droplets or washed blood are found at crime scene. If there is no victim or suspected body found, the time of estimation of blood pool formation becomes a critical section of information. The dynamics of evaporation of blood pool establishes new aspects of fluid mechanism. There are five distinct stages of evaporation of blood pool is seen with distinct morphological characteristics. These stages include; Coagulation stage, Gelation stage, Rim desiccation stage, Centre desiccation stage, Final desiccation stage[12]. The pool of blood does not dry in a uniform manner; it depends on the dynamics of evaporation. Other than the evaporation aspect, fibrins have a major role in the drying mechanism of blood pool.

The rate of evaporation depends on the environmental conditions (such as temperature, humidity, and air flow), size of pool, target surface and its porosity. Several studies have been performed to estimate how these and other factors affect the drying of blood to better understand the deportment of blood at crime scene[13]. The drying of blood begins approximately 50 seconds after it has been deposited. The area of spread over droplet of blood will depend on certain physical properties such as type surface and viscosity of fluid, on these factors the surface tension and angle of contact will depend. The surface tension (γ) can be defined as the amount of energy which is required to increase the surface area by one square meter. In other words, for increasing the area of impact of a droplet requires the energy and more energy is required i.e. higher is the surface tension[14].

By deducinga droplet or pool spread upon a surface into young's equation:

$$\mathbf{S = \gamma (\cos \theta - 1)}$$

Here, S is known as the spreading parameter,

γ is the surface tension between gas and liquid interface and θ is the angle of contact between liquid and the surface, When the angle of contact is much smaller than 90◦ (S is positive) the surface wetting, i.e., the liquid can easily spread over the surface and the surface is supposed to be wet. When the contact angle is much larger than 90◦ (S is negative)

the liquid cannot spread over the surface easily and the surface is supposed to be not wet. Angle of contact has a significant role in the drying of the blood pool.

Blood pool will cover larger surface area when the angle of contact is small. Drying of a blood pool works on the principle of evaporation. Drying of a blood droplet starts from the periphery and centre of droplet is the last part. Cracks were formed when the droplet starts drying. Drop of blood dries following two distinct regimes and by going through five different stages[14]. First regime, the evaporation of the liquid starts taking place. The RBS's are evenly distributed in the blood droplet but when the evaporation takes place the solvent starts evaporating by inducing evaporation flux at the interface and hence the gel is formed due to the high concentration of cellular component inside the blood droplet. The rim will dry first due to the internal flow of transporting elements inside the drop. Second regime, is quite slow as it is diffusive in nature[15]. The actual drying and formation of cracks can be seen at this regime. The desiccation starts from the periphery of the drop and dries towards the centre of the drop. The height of the pool starts decreasing as the drying is accomplished. In case of blood pools the volume of blood is large so that the gravitational forces will dominate over the surface tension forces producing a flat surface on top of the pool[16].

In the first stage, blood is liquid by nature and starts coagulating by the action of fibrinogen. The colour of the blood pool changes from dark red to lighter red. The surface area of the blood pool increases as the angle of contact is smaller than 90◦. That's why, this stage is known as coagulation stage. In the second stage, evaporation starts to take place. The transition from the fluid to gel is noticed and hence referred as gelation front. This stage starts when the gelation rim is formed around the pool[17]. In the third stage, rim starts to change its colour from red to black and this is called as rim desiccation. The drying front is observed by the transition of red colour rim to black colour rim. During this stage the drying of rim is seen apart from this gelation is seen at the centre[18]. This stage is known as rim desiccating stage. In the fourth stage, once the gelation front reaches the centre of the bloodstain, the entire stain has attain the gel stage. The drying front and cracks generate towards the centre of the stain. This stage is known as centre desiccation stage. In the Final stage, the drying front reaches the centre of the blood pool. The pool has almost completely desiccated. During this stage, the entire pool changes from red to black in colour. Lastly, the liquid evaporates, the remains diminished and the cracks reach the centre of the stain.

Body fluid microbiome

At crime scenes, the most commonly found body fluids are blood, semen and saliva. However, other fluids (e.g., vaginal fluid, urine and sweat) can also be present and perform meaningful roles in investigations, namely as DNA evidence[19].It has been proposed that each each body fluid type presents a specific composition of microorganisms that can be used as bioindicators, being possible to infer the type of body fluid present based on its microbial composition. For instance, saliva could be identified by detection of specific bacteria, such as Veillonella atypical, Streptococcus mutants and Streptococcus salivarius; while vaginal fluid could be identified by detection of specific bacteria, such as Lactobacillus crispatus and Lactobacillus gasseri. When considering lower taxonomic levels (i.e., genus, species and strains), the human microbiome is utterly characteristic from a given individual. Therefore, the finite solution of the results acquired from MPS-based techniques will allow identifying suspects comparing the micro biomes or at least theoretically will help to establish an association between perpetrators and victims[20]. Conclusions Over the preceding decade, the advances in MPS and related techniques have revolutionized science in many numerous fields, from individualized medicine to forensics. Now a days, it is possible to sequence complete genomes or complex samples in a few hours at an affordable price. As such, these innovative techniques can be used as a complement to classical microbiological methods in a polyphasic approach.

As it has been previously suggested that one of the most prominent breakthroughs associated with microbial forensics might be reached in the cases of sexual offenses, associating offender and victim even several years after the crime has been committed and using as bioindicators sexual transmitted dis-eases (e.g., HIV and HCV). This possibility was proven in a case in which a step-father was convicted of abusing his 10-year-old stepdaughter, based on the phylogenetic analysis of HIV transmitted[21]. However, as previously referred, prior to its wide

implementation in forensic investigations, several steps should be considered. Indeed, it is necessary to define standard operational protocols for sample col-lection, handling and preservation. All methods should be further validated, concerning aspects such as sensitivity, specificity, reproducibility, repeatability and limit of detection. Finally, the creation of reliable and comprehensive databases is mandatory for the accurate interpretation of the obtained results.

DISCUSSION

In general, fungi have a very short life span though it differs greatly from species to species. Some types may live as short as a day while others survive anywhere between a week and a month. The life cycle of a fungus begins as a spore and lasts until germination. Transportation and storage of the samples at room temperature encourages the growth of mesophilic bacteria and fungi in them, thus increasing their chance for degradation[22]. Additionally, water activity and the relative humidity of air in equilibrium with a sample play an important role in microbial degradation of biological samples. It has been well evidenced that the higher water activity of the biological samples enhances their chance of degradation as high water activity (aW) promotes the microbial growth. Microbial By-Products and Associated Problems for DNA Typing Micro-organisms contaminating the biological samples from any of the previously described routes are capable of encumbering the DNA typing analysis. DNA degradation or damage is one of the major causes to obtain null result in DNA typing techniques.

DNA damage may occur by missing nucleotide base, altered base, incorrect base, bulge due to the deletion or insertion of a nucleotide, linked pyrimidines, single. Microbial metabolites and their effect on DNA typing techniques Compounds Effect Source Reference Dimethyl sulphide (DMS)Mutation in DNA sequence by alkyla ting activity Pseudomonas putida and Pseudomonas deceptionensis N-methyl-N-nitrosourea (MNU) Mutation in DNA sequence by alkylating activity Pseudomonas aeruginosa. Reactive oxygen species (ROS)Formation of single stranded breaks (single stranded nicks)Enteric commensal bacteria, Nucleases Strand breaks Staphylococcus aureus, Bacillus lichen informs, Proteases Degradation of polymerase enzyme inhibiting PCR process Pseudomonas aeruginosa, Streptomyces griseus, Staphylococcus aureus and Aerococcus viridians, Hydrolytic agents Incorporation of erroneous bases during amplification and change of coding Trichoderma harzianum, few genera of gamma-proteobacteria, Cell debris (proteins, polysaccharides, salts)Degradation/sequestration of nucleic acids Inorganic ions Reduction of specificity of primers and degradation of polymerase any microbial contamination, Exogenic DNA Competition with template.

SOURCE OF FUNDING: NA

CONFLICT OF INTEREST: NA

ETHICAL CONSIDERATION: NA

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Table No.1: life span of growth of bacteria and fungi over blood samples.

Sample	Potent group of Bacteria	Life span of Bacteria	Potent group of Fungi	Life span of Fungi
Blood	Yersinia enterocolitica, Staphylococcus epidermidis, S. aureus, Bacillus cereus, Propionibacterium acnes, Serratia spp, Streptococcus spp.,	Bacteria divide somewhere between once every 12 minutes and once every 24 hours. So the average lifespan of a bacterium is around 12 hours or so.	Mucor sp., *Penicillium sp., Aspergillus sp.*	Fungi have a very short life span, though it differs greatly from species to species. Some types may live as short as a day, while others survive anywhere between a week and a month.

RESEARCH ARTICLE

Co-Inoculation Effect of Liquid Formulation of *Rhizobium* and Phosphobacteria on Growth and Yield of Groundnut (*Arachis hypogea* L.)

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ABSTRACT

A pot culture experiment was carried out in the Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram to study the Co-inoculation effect of a liquid formulation of *Rhizobium* and Phosphobacteria on the growth and yield of groundnut (*Arachis hypogea* L.).The experiment was carried out using a completely randomized design (CRD) with three replication. The bio inoculants viz., *Rhizobium* sp., and *Bacillus megaterium* (phosphobacteria) were used in this experiment to enhance the growth and yield of groundnut by fixing atmospheric nitrogen and solubilizing phosphate in soil (to make available) respectively. Apart from they are producing plant growth hormones like (Indole acetic acid, Gibberellic acid, and Cytokine), In this experiment, the following treatments *viz* T1-control, T2- RD (Recommended Dose) NPK, T3-75%N RD with *Rhizobium*, T4-75% P_2O_5 RD with phosphobacteria, T5-75% N and P_2O_5 RD with *Rhizobium* and phosphobacteria, T6-50% N RD with *Rhizobium*, T7- 50% P_2O_5 RD with Phosphobacteria , T8-50% N and P_2O_5 RD with Rhizobium and Phosphobacteria were adapted. Among the treatments, treatment T4 recorded higher plant height, shoot length, dry matter production, No. of pods per plant, nuts yield, and stalk yield followed by treatment T7-(50% N and P_2O_5 RD with *Rhizobium* and Phosphobacteria). The present study

revealed that 25% nitrogen and 25% Phosphatic fertilizers can be saved. The study observed a positive interaction between the *Rhizobium* strains and phosphobacteria.

Keywords: *Rhizobium*, PSB, groundnut, nitrogen,liquid formulation.

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an essential food legume in tropical and subtropical areas because of its capacity to adapt to a wide range of agro-climatic regions. Groundnut is normally cultivated in nutrient-poor soil and rain-fed conditions, so the average yield becomes to be very low relative to potential yield. Even though the nitrogen (N) need of groundnut is much higher than cereals owing to its high protein content, it has the ability to meet 60-80% of N-based requirements due to symbiotic N fixation via its root nodules. In its symbiotic relationship with legumes, *Rhizobium* fixes N, thereby positively influencing the content of this nutrient. After nitrogen, phosphorus (P) is an important mineral fertilizer for plant growth and development and is the world's second-largest agricultural chemical. Phosphorus is one of the three macronutrients that are necessary for plant growth and development. The use of phosphate solubilizing bacteria can determine to be an important measure to supply phosphorous to the groundnut plants to enhance productivity. Phosphate solubilizing bacteria (PSB) play an essential role in reducing P scarcity in the soil through transforming insoluble phosphate to available and soluble phosphate. In return, the plant gets the fixed N from the nodules and produces food and forage protein. Inoculation of legumes with rhizobia generally triggers plant growth, development and yield and it is normally used as a substitute for mineral nitrogen fertilizer which is often costly (Tairo and Ndakidemi, 2013). The rhizobia (bacteria) have the potential to infect the root, form nodules and symbiotically fix N2 in leguminous plants (Kevin Vessey, 2004). However, the *Rhizobium* is host-specific as certain species can only infect specific legumes. Liquid inoculant formulation is one solution to the problems associated with processing of solid carriers. The use of various broth cultures amended substance that promotes cells survival in the package and after application for seed (or) soil. Additives to liquid inoculant formulations should have a role in protecting microbial cells on seed at high temperature and during desiccation. Many kinds of polymers have been used for inoculant production because of their ability to limit heat transfer, their good rheological properties and high water activities (Mugnier and Jung, 1985; Kumaresan and Reetha, 2011). Studies showed enhanced IAA synthesis, more seed germination and overall improvement in plant growth by well-known that Phosphate solubilizing bacteria (PSB) and Rhizobium have synergistic effect on crops.

MATERIALS AND METHODS

A pot culture experiment was conducted at the Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai university, Annamalai nagar, Chidambaram .to study the effect of Coinoculation effect of liquid formulation of rhizobium and phosphobacteria on growth and yield of groundnut(*Arachis hypogea* L.). The experiment were carried out in a completely randomized block design with three replications and eight treatments. The treatment were applied T_1-control, T_2- RD NPK, T_3-75%N RD with rhizobium,T_4-75% P_2O_5RD with phosphobacteria,T_5-75% N P_2O_5RD with *Rhizobium* and phosphobacteria,T_6-50% N RD with *Rhizobium*, T_7- 50% P_2O_5RD with Phosphobacteria ,T_8-50% N and P_2O_5RD with *Rhizobium* and Phosphobacteria. The soil having a pH of 8 organic carbon 0.54%, available N, P2O5 S and K_2O were 254,35 and 335 kg/ha respectively. Recommended fertilizer (25:75:25 kg of N: P_2O_5: K_2O) were supplied in the farm of urea, DAP, MOP as in 2 splits. Further, The following growth, yield parameters were studied for plant height, root length, dry matter production, Filled Pods ,Total pods ,Pod weight, Pod yield , Haulm yield ,total kernel weight. The surface sterilized groundnut TMV-2 seeds were coated with the inoculum of liquid formulation of *Rhizobium* sp. and phosphobacteria (*Bacillus megaterium*) at the rate of15 ml kg^{-1} with equal volume of sterile water and rice gruel were inoculated directly and inoculated seeds were sown in cement pots (1' × 2' × 2') containing sterilized soil at the rate of five seeds pot^{-1}. The remaining seeds were aseptically kept at room temperature for *in vitro* assessment on the survival studies.

Observations on growth parameters
Five plants from each treatment were randomly selected for recording growth parameters periodically at 30 and 60 days after sowing (DAS) and at harvest.

Effect on plant height
The plant height was measured from the base of the plant to fully opened top leaf and expressed in cm.

Effect on dry matter production
Three plants from each pot were randomly selected, uprooted carefully and thoroughly washed on 30 DAS. Then the samples were dried in hot air oven at 80°C until a constant weight was obtained. The dry matter production was calculated and expressed in grams per plan.

Pod Yield
Plants in each pot were harvested and the pods picked from the roots and dried in the sun on a concrete floor for a sufficient number of days and then weighed using a digital balance.

100-Seed Weight
In order to determine the average seed size/weight, about 100 pods were randomly selected per treatment, shelled and the seeds were mixed and from this number 100 seeds were picked and weighed to estimate the average seed size/weight.

Haulm Yield
After removing the pods from the plants harvested from the each pot, the leaves and the roots were removed from the plants and the remaining stuff was weighed and recorded as field weight. In order to determine the moisture content of each pot of haulm, a sub-sample of the haulm was taken from each treatment, weighed and oven-dried at 60°C to a constant weight. , the percent moisture content of the sub-sample and the field weight of the bulk haulm were used to calculate the dry weight of the bulk haulm.

Assessing the survival of inoculated *Rhizobium* and phosphobacteria in rhizosphere soil
The survival of inoculated *Rhizobium* and phosphobacteria in rhizosphere soil was estimated by MPN method on 30 and 60 DAS and at harvest.

RESULT AND DISCUSSION

Coinoculation effect of liquid formulation of *Rhizobium* and phosphobacteria on growth and yield parameters of groundnut (*Arachis hypogea* L.)
The present research work to study the combined effect of liquid formulation of *Rhizobium* and phosphobacteria on growth parameters *viz*., plant height, and root length, and dry matter production of groundnut.

Plant growth parameters
The values of different plant growth parameters like plant height, root length and dry matter production are presented in table I. The plant height was observed on 30, 60, and 90 days after sowing (DAS) and at harvest in ground nut. The plant height was recorded with inoculation of *Rhizobium* and phosphobacteria were higher when compare to uninoculated treatments. The Treatment T_5 (75% N and P_2O_5 RD with *Rhizobium* and phosphobacteria) augmented higher plant height followed by T_8 (50% N and P_2O_5 with *Rhizobium*+ phosphobacteria). The root length and dry matter production were recorded increasing values from 30 DAS to at harvest stage. The treatment T_5 (75%N and P_2O_5 RD with *Rhizobium* and phosphobacteria) were observed higher root length and dry matter production at harvest (9.5 cm and 32.01 g per plant) over the control (without inoculation). The control treatment was recorded lowest root length and dry matter production at 30 DAS (2.5 cm and 6.25 g per plant) respectively

when compared to all treatments. These findings are similar with that of Bansal, (2015), they reported higher plant height, root length and dry matter production in mungbean (*Vigna radiata*). The highest plant growth parameters were obtained due to nitrogen fixation and plant growth hormones production by *Rhizobium* and conversion insoluble phosphate into soluble form phosphate by secretion of organic acids and phosphatase and phytase enzyme through phosphobacteria.

Yield parameters

Inoculation of groundnut with liquid formulation of *Rhizobium* and phosphobacteria and different graded levels recommended dose (RD) of nitrogen and phosphorus were significantly increased yield parameters *viz*., Filled pods plant^{-1},total pods plant^{-1}, Pod weight plant^{-1} (g) , Pod yield (kg ha^{-1}) , Haulm yield (kg ha^{-1}) and 100 kernel weight(g) in table -II. An application of 75%N and P_2O_5 RD with *Rhizobium* and phosphobacteria produced the highest filled pods per plant, total pods per plant, pod weight per plant and 100 kernal weight (22.8, 31.4, 22.4 and 45.2 g) respectively, While the lowest value yield parameters obtained in T_1 (control- without inoculation bioinoculants). According to Ndakidemi *et al*., (2006), the combined use of P fertilizers and *Rhizobium* inoculants increased grain yield and enhanced N2 fixation to improve the fertility status of most soils. Present study is in agreement with this finding as the combined use of P and inoculants increased yields of the test groundnut varieties. Application of 75% N and P_2O_5 RD with liquid formulation of *Rhizobium* and phosphobacteria produced the highest pod and haulm yield of 2823 kg per ha, 4656 kg per ha respectively when compared to control (T1). The result obtained is in agreement with the work done by Zoundji *et al*., (2015) who reported that combined applications of Phosphobacteria and *Rhizobium* inoculant enhanced pod and haulm weight of soybean and other legumes.

CONCLUSION

The present investigation confirms that combined inoculation of liquid formulation of *Rhizobium* and phosphobacteria strains were increased the growth and yield parameters of groundnut rather than single inoculation. As a result of co-inoculation of both the organisms can be saved 25 % of inorganic nitrogen and phosphophatic fertilizers.

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Table I. Coinoculation effect of Liquid Formulation of *Rhizobium* and Phosphobacteria on Growth Parameters of Groundnut

T.No	Treatment	Plant height (cm)			Root length (cm)			Dry matter production/ plant (g)		
		30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest	30 DAS	60 DAS	At harvest
T_1	Control	15.08	17.87	20.87	2.5	5.8	7.6	6.25	14.23	19.98
T_2	Recommended Dose (RD)NPK	16.02	20.06	27.98	2.7	5.9	7.9	6.42	15.26	20.04
T_3	75%N RD with *Rhizobium*	17.28	25.06	32.76	4.3	7.2	9.1	7.85	17.06	29.02
T_4	75%P_2O_5RD with Phosphobacteria	17.08	25.05	30.54	3.7	6.9	8.4	7.77	16.09	26.28
T_5	75%N P_2O_5 RD with *Rhizobium* and Phosphobacteria	19.02	27.02	39.01	5.2	7.8	9.5	8.62	19.09	32.01
T_6	50%N RD with *Rhizobium*	17.03	23.08	29.09	3.2	6.8	8.1	7.63	16.07	22.02
T_7	50%P_2O_5RD with Phosphobacteria	16.86	21.87	296	2.8	6.2	8.0	6.98	15.84	21.54
T_8	50% N and P_2O_5 with *Rhizobium*+ phosphobacteria.	18.76	26.08	35.08	4.8	7.4	9.3	8.23	17.02	29.06
	CD	**0.39**	**0.79**	**3.32**	**0.4**	**0.35**	**0.32**	**0.32**	**1.05**	**1.55**
	SEm±	**0.16**	**0.37**	**1.62**	**0.1**	**0.16**	**0.15**	**0.15**	**0.62**	**0.75**

Table II. Co inoculation effect of Liquid Formulation of *Rhizobium* and Phosphobacteria on Yield Parameters of Groundnut.

T. No.	Treatments	Filled Pods plant^{-1}	Total pods plant^{-1}	Pod weight plant^{-1} (g)	Pod yield (kg ha^{-1})	Haulm yield (kg ha^{-1})	100 kernel weight(g)
T_1	Control	18.7	27.3	17.6	2052	4061	39.2
T_2	RD NPK	19.2	28.7	18.1	2173	4162	41.5
T_3	75%N RD with *Rhizobium*	21.3	30.3	21.8	2573	4446	43.9
T_4	75% P_2O_5RD with Phosphobacteria	20.0	29.9	20.06	2447	4321	43.6
T_5	75%N P_2O_5 RD with *Rhizobium* and phosphobacteria	22.8	31.4	22.4	2823	4656	45.2
T_6	50% N RD with *Rhizobium*	19.8	29.4	20.4	2376	4302	42.7
T_7	50% P_2O_5 RD with phosphobacteria.	19.6	29.2	19.02	2362	4286	41.7
T_8	50%N and P_2O_5 RD with *Rhizobium* and phosphobacteria.	22.1	30.8	22.2	2656	4632	44.7
	CD	**0.675**	**0.65**	**0.95**	**106.75**	**66.5**	**1.02**
	SEm±	**0.34**	**0.32**	**0.47**	**53.27**	**34.15**	**0.5**

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RESEARCH ARTICLE

A Survey on Different Techniques for Fake News Detection

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ABSTRACT

The proliferation of fraudulent news is a severe issue that can hurt society. In the recent era, it has been discovered that more people obtain news via search engines and social media than traditional media such as print media. However, there is no way to verify the accuracy of the available information online. Given the difficulties in recognizing fraudulent news, many researchers attempt to grasp the issue statement and its characteristics. The challenges and limitations of fraudulent news determination studies were discussed in general. Overall, this study brings a thorough introduction to the topic of automated fraudulent news determination, which will aid future research efforts in this area.

Keywords: Fraudulent news, social media, search engines, previous techniques, and authenticity.

INTRODUCTION

Assuring the accuracy of the information has become a critical issue with severe consequences for society. Because a rising number of individuals rely on social media to acquire information and news, news providers no longer have control over the flow of information. As a result, there is an urgent need to ensure that the news presented online is authentic. Because social media can reach millions of people in seconds, disinformation can readily spread without actively monitoring [1,2,3,4,5]. Others can use it to further their agendas or goals in that instance. Therefore it necessitates careful care and regulation. Fraudulent news can have a big impact[6,7,8,9]. It harmed the stock market in 2013. The stock market lost 130 billion dollars after the false report that an explosion hurt Barack Obama. Fraudulent news had the most impact on the US presidential elections [10,11,12,13,14]. Though a study shows that fraudulent information did not influence election results, the issue gained attention because roughly 20% of voters indicated that social media news influenced their choice of candidate[15,16,17,18,19]. Fraudulent news is purposefully false information that can deceive anyone. Fraudulent news determination is a developing research

challenge [20,21,22, 23, 24,25]. Many academics are trying to differentiate fraudulent information from real information, and this sector has seen a growth in researchers. Although many procedures and frameworks have been recommended, efficient automatic fraudulent information determination remains challenging [26,27]. This study reviews traditional strategies employed in current fraudulent news determination research, comparing the procedures at each stage. Discuss the general issues and limitations of fraudulent information determination studies. Overall, this paper gives a thorough introduction to automated fraudulent information determination, paving the way for future research on this topic.

Related works

A few technical issues must be addressed in solutions. For starters, computational-oriented fact-checking does not cover all of the relationships required for fraudulent information determination. Second, validate the validity of the retrieved triples from news items. Pan et al. [2018][28] offered a simple way to recognize fraudulent news based on news content utilizing knowledge graphs, including the B-TransE technique. These techniques are tested using Kaggle's 'Getting Real about Fraudulent News' dataset and some genuine magazines from mainstream media. According to the findings, some techniques have F1 scores of more than 0.80. Jain *et al.* [2019][29] present a technique for recognizing fraudulent information. The author attempted to collect information using ML and natural language processing and then use SVM to decide if the information was true or false. The recommended technique's findings are compared to those of existing processes and are effective, defining the correctness of outcomes up to 93.6 %. Mandical *et al.* [2020][30] recommended classifying bogus news. The difficult work of recognizing fraudulent news can be simplified by using the appropriate techniques and instruments. ML procedures such as Naive Bayes, Passive Aggressive Classifier, and Deep Neural Networks were employed on 8 datasets obtained from multiple sources.

Each technique's analysis and findings are also included in the publication. Mahabub[2020][31] recommended an Ensemble Voting Classifier-based intelligent determination technique for both authentic and fraudulent news categorization. In Ensemble Voting Classifier, we used the best three ML techniques. These include Naive Bayes, K-NN, SVM, Random Forest, ANN, Logistic regression, Gradient Boosting, and Ada Boosting. The testing results show that the recommended framework can achieve 94.5 percent accuracy. The recommended determination system can successfully locate the news's key points. False profiles, false messages, etc., can be recognized using these. Bhutani *et al.* [2019][32] recommended a new approach for fraudulent news determination that includes sentiment as a critical component for increasing accuracy. It also evaluates the recommended technique's efficiency using three different data sets. The results indicate that the recommended solution works well. (Kotteti *et al,* Jaafar *et al,* Ghorbani *et al,* Kumari and Ekbal, Li *et al,* Saikh *et al*) introduced a novel data pre-processing approach to analyze missing values [33,34,35,36,37,38,39]. Furthermore, TF-IDF vectorization is used in feature retrieval to remove inappropriate characteristics. Experiment findings show that using the recommended data preprocessing strategy with a Multi-Layer Perceptron (MLP) classifier surpasses baselines and boosts prediction accuracy by more than 15%. Elhadad *et al.* [2019][40] attempted to technique how humans interact with news documents in real life.

Introduced a new technique for dealing with the entire textual content of news magazines by retrieving several textual attributes and a sophisticated set of other metadata-related aspects without segmenting the news documents into portions (title, content, date, source, etc.). The efficiency of 9MLproceduresregarding accuracies, precision, recall, and F1-score are examined, yielding superior results than previous efforts. [Singhal *et al.,* Mangal and Sharma, Shah and Kobti, Palani *et al.,* Qiet al, Giachanou *et al.*] [41,42,43,44,45,46] developed Spot Fraudulent, a multi-modal framework for spam filtering. The recommended technique recognizes bogus news without considering any other subtasks. Text characteristics are learned using language techniques (such as BERT), and image characteristics are known using VGG-19 pre-trained on the Image Net dataset. All tests are carried out by using Twitter and Weibo. On Twitter and Weibo datasets, the conceptual technique exceeds the existing techniques by 3.27 % and 6.83 %, respectively. Reis *et al*. [2019][47] introduced a new set of characteristics and assessed the forecasting efficiency of existing techniques and characteristics for automatic determination of fraudulent news. The findings offer intriguing insights about the usefulness and significance of attributes for recognizing bogus information. Finally, consider how fraudulent news determination systems might be employed in practice, emphasizing obstacles and potential.

[Kaur *et al.,* Aphiwongsophon and Chongstitvatana, Al-Ash and Wibowo, Ghosh *et al,* Anjum *et al.*] recommended a new multi-level voting ensemble technique [48,49, 50,51, 52].

The recommended technique can also predict false information (in textual form) from online social media platforms. Using twelve classifiers, the recommended system was evaluated on three datasets. Based on their efficiency metrics, the Passive Aggressive, Logistic Regression, and Linear SVC techniques carry out best using TF-IDF, CV, and HV feature retrieval techniques, respectively. In contrast, the recommended strategy exceeds the Passive-Aggressive process by 0.8%, the Logistic Regression technique by 1.3%, and the Linear SVC technique by 0.4% using TF-IDF, CV, and HV, respectively. Ozbay and Alatas [2020][53] developed a two-step technique for spotting fraudulent information on social media has been, focusing on fraudulent information. In the initial step of the approach, several preprocessing is applied to the data set to convert unstructured data sets into structured data sets. In the next step, twenty-three supervised artificial intelligence procedures are established in the data set processed into the structured format with the text mining techniques. Shabani and Sokhn [2018][54] recommended a hybrid machine-crowd strategy for recognizing possibly misleading news. This system combines human input with ML and a decision-making technique that evaluates algorithmic categorization confidence and decides whether human input is required. This technique yields comparable accuracy to the stated baseline values but at cost and delay. Schütz *et al.* [2021][55] introduced a binary content-based categorization strategy for recognizing fraudulent news automatically using pre-trained language techniques based on the Transformer architecture. We used XL Net, BERT, RoBERTa, Distil BERT, and ALBERT on the Fraudulent News Net dataset, with various hyper parameter combinations. These levels used only the body text, titles, or a mix of the two. Since Transformers can recognize bogus news without utilizing a vast dataset, they are a potential technique to recognize fraudulent news. Our essential contribution is to improve the determination accuracy of fraudulent information by using various techniques and parameterizations with a reproducible outcome examination. A short paragraph can achieve 85% correctness on the test set—up to 87 percent accuracy. Finally, it shows that preprocessing measures like eliminating outliers do not affect the technique's prediction output.

Yuan *et al.* [2021][56] recommended a "domain-adversarial and graph-attention neural network" (DAGA-NN) technique. Extensive testing on Twitter and Weibo multimedia datasets revealed that the recommended procedure effectively-recognized fraudulent news across events/domains. Its main benefit is that in a text environment with several events/disciplines, just partial domain sample data is required to train the technique for accurate cross-domain fraudulent information determination in fields with few (or no) samples (where there is no sample data).
[Granik and Mesyura, Mahir *et al.,* Al-Ash et al., Abdelminaam *et al.*] [57,58,59, 60]recommended a simple strategy for fraudulent news determination using NB classifier, and was tested on a set of Face book news posts. Considering the technique's relative simplicity, we achieved a categorization accuracy of roughly 74% on the test set. The results show that artificial intelligence can recognize bogus news. Several techniques are presented in the article to improve these findings.

INFERENCES FROM THE EXISTINGWORK

Due to their cost-effective, quick access, and rapid dissemination, social media are becoming a primary news source for millions worldwide. This comes at the expense of doubtful credibility and exposure to 'fraudulent news written to mislead readers. Fraudulent news determination has always relied on spreader profiles and the dissemination structure. However, data collecting is complex, and early determination of bogus news is impossible. Another technique is to recognize fraudulent news purely by its substance. In the past, linguistic characteristics were manually created. Domain-specific shpermit characteristics cannot be easily adapted to cross-domain data.

SOLUTION

The idea of building capsule neural networks for fraudulent news determination is being explored. Also, it introduces multiple embedding techniques for different news magazines. Static word embeddings are utilized for short news items, while non-static word embeddings permit incremental training and upgrading during the training level. The n-gram level for feature retrieval will also vary.

CONCLUSION AND FUTURE WORK

This study describes fraudulent news determination technologies and how they have been implemented for determination. According to research, each of the strategies presented has its strengths and shortcomings, and there is no single technique for recognizing fraudulent news. Finally, the open research issues in the fraudulent news determination sector are addressed by analyzing the benefits and cons of existing systems. As a result, it is concluded that future work in this field would concentrate on constructing capsule neural networks to recognise fraudulent news. In addition, multiple embedding techniques for news items of varying lengths are introduced.

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Table:1.Comparison of existing techniques for fraudulent news determination

Author name	Techniques	Merits	Demerits
Granik and Mesyura [2017]	Naive Bayes	Provides better efficiency	Lesser accuracy
Jain et al. [2019]	Support vector machine	Obtains 93% accuracy	Need to enhance the efficiency and accuracy of the prototype
Mandical et al. [2020]	Naive Bayes, Passive Aggressive Classifier	Achieves higher accuracy	Times consuming nature
Mahabub[2020]	Ensemble Voting Classifier	Produces higher accuracy	It cannot help unknown differences between sample and population
Kotteti et al. [2018]	Multi-Layer Perceptron	Outperform baselines	the recommended technique does not test on other data sets for fraudulent news determination
Singhal et al. [2019]	Spot Fraudulent-a multi-modal framework	Performs better than the existing technique	Very expensive
Kaur et al. [2020]	Multi-level voting ensemble	Accurate prediction results	New observations are still confuse
Shabani and Sokhn [2018]	Hybrid machine-crowd technique	Achieves reasonably higher accuracy	Time-consuming nature
Schütz et al. [2021]	Binary content-based categorization	Reach up to 87% accuracy	Very expensive
Yuan et al. [2021]	Domain-adversarial and graph-attention neural network	Surpassed existing technique baselines	Lesser accuracy

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RESEARCH ARTICLE

Clathrate Hydrate Crystals and Charge Transfer Interaction Characterize High Dilutions of Two Homeopathic Drugs *Cannabis sativa* and *Colchicum autumnale*

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ABSTRACT

High dilutions (HD) of drugs used in homeopathy are mostly devoid of original drug molecules. Water structure has been reported to carry the information of original drug molecules. We have defined the water structure in terms of free water molecules, hydrogen bond strength and number of hydrogen bonds. We have also reported that charge transfer interaction (CT) has been associated with HDs. In the present study we have analysed two drugs *Cannabis sativa* and *Colchicum autumnale,* and two potencies 6cH and 30cH by electronic and vibrational spectroscopy. The UV-Vis spectra of each potency show two peaks one at 200 nm and another 220 nm wave length. The first peak belongs to the absorbance by clathrate hydrate crystal (CHC), the second peak at higher wave length has been assigned to the CT interaction. In the CT interaction dissolved oxygen serve as electron acceptor and water or ethanol as electron donor. Dissolved oxygen might have been introduced in the solvent medium (EtOH-water) of the potencies during their preparation by mechanical agitation or succussion. CT interaction appears to

be a common factor for all homeopathic potencies. We have quantified free water molecules in the potencies from FTIR-spectra in the wave number region 3700 cm^{-1} to 3550 cm^{-1}.

Keywords: Homeopathic drug, High dilutions, Free water molecules, Charge transfer, UV Spectra, FTIR-spectra.

INTRODUCTION

Homeopathy uses extremely high dilutions (HD) of drugs prepared by serial dilution followed by mechanical agitation or succussion (Hahnemann, 1833a; Hahnemann, 1833b; Sukul and Sukul 2004). HDs thus prepared are called potencies designated as 6 cH, 12 cH, 30 cH, 200 cH etc. Potencies from 12 cH onward do not contain the original drug molecules, but they vary from each other with respect to their therapeutic and biological effects (Sukul and Sukul, 2004; Sukul *et al.*, 2001; Hahnemann, 1833a; Hahnemann, 1833b). Water structure in potencies is thought to be responsible for the physico-chemical basis of potentized drugs in absence of original drug molecules. We have already reported that free water molecules, number of hydrogen bonds, hydrogen bond strength and charge transfer interaction (CT) contribute to the variation of water structure in these potencies (Ghosh *et al.*, 2021; Singh *et al.,* 2021). In the present study we report the CT interaction and quantities of free water molecules in two potencies 6 cH and 30 cH of two drugs *Cannabis sativa* and *Colchicum autumnale* by electronic and vibrational spectroscopy. CT interaction plays an important role in many biological processes such as enzyme actions, DNA base pair binding etc. (Ghosh *et al.*, 2008; Fujitsuka and Majima. 2013; Zhang *et al.* 2014; Sjulstok *et al.* 2015; Lienemann 2020).

MATERIALS AND METHODS

Drugs

Two potencies, 6 cH and 30 cH of *Cannabis sativa* and *Colchicum autumnale* were used in the experimental study. Dried inflorescence with a few leaves of *C. sativa* was purchased from a retail shop under the controller of Excise Department, Govt. of India at Bolpur, Birbhum, West Bengal. The material was extracted with 90% EtOH (Merck, Germany, Lot No.-K51652783934) at room temperature for 4 days. The MT of *C. autumnale,* a product of Dr. Reckeweg & Co. GmbH, Germany (Col MT Lot No.-2989IN370091) was purchased from a local market at Kolkata. Both the MTs were diluted with 90% EtOH 1:100 (v/v), and their Optical Density (OD) was measured with a UV-VIS spectrophotometer (SHIMADZU, Model-UV-VIS 1900i, Software- Lab solutions UV-VIS) at room temperature.(24±2) at a wavelength of 200 nm to 300 nm. The OD for *C. sativa* and *C. autumnale* was 0.12 and 0 03, respectively. Each MT was diluted with the deionised and distilled (DD) water and succussed manually 10 times to prepare the I^{st} centesimal potency called 1 cH following the standard method of potentization of Homeopathic drugs (Sukul and Sukul 2004). Using the same procedure of successive dilution and succussion we prepared 6 cH and 30 cH of the two drugs. The 6cH and 30cH potencies were preserved in 90% EtOH. Each potency was further diluted with DD water so as to reduce the percentage of EtOH to 20 for further analysis by UV and FT-IR spectroscopy. At 20% EtOH hydrogen bond strength is highest (Burikov *et. al.,* 2010)

UV-spectra

UV-spectra of all the test samples were obtained in the wavelength 200-300 nm at medium scan speed and 0.5 nm data interval using a UV-VIS spectrophotometer. The baseline was kept at 20% EtOH because all the test samples were in 20% EtOH. Five spectra of each sample were averaged. The margin of error (MOE) was calculated from the following formula, MOE = $Z^* \times \sigma/\sqrt{n}$ where Z^* = Z score, σ = SD, n = number of samples (Banarjee PK, 2004; Rumsey DJ, 2016).

FT-IR –spectra

FT-IR spectra of all the test samples and their control (blank 20% and 90% EtOH) were obtained using Shimadzu IR Affinity -1S Fourier Transform Infrared spectrophotometer (Spectrum two) on the attenuated total reflection (ATR) technique. The energy resolution was 0.5 cm^{-1}. The baseline was corrected for atmospheric humidity and CO_2. One drop of each sample was put in the sample groove, and the tip of a single reflection pure diamond crystal was brought in contact of the sample drop to record the whole spectrum in the wave number range of 4000 to 500 cm^{-1}. Forty-five scans were averaged to improve the signal to noise ratio. The ratio of absorbance intensity (A) at 3240 cm^{-1} (strong hydrogen bonding) and 3360 cm^{-1} (weak hydrogen bonding) was determined in order to quantify contour shape of the OH-stretching band (Burikov *et al*., 2010). For OH bending (v_2) band the ratio was calculated between A 1580 $cm^{-1}$and 1690 cm^{-1}. The ratio values were calculated after normalization of FT-IR spectra. During the experiments the room temperature and humidity were 24^0C and around 50%, respectively. We have also compared the FTIR spectra of the test samples at 90% and 20% EtOH in the wave number region of 3700-3550 cm^{-1}. This is because in this range free OH groups appear (Mulliken, 1955; Tamer *et al*., 2014; Tamer, 2017).

RESULTS

UV-spectra

The spectra of the two potencies 6 cH and 30 cH of two drugs *Cannabis sativa* and *Colchicum autumnale* are presented in Figures 1 and 2, respectively. In both cases the absorbance was positive showing two distinct peaks. *Cannabis* 6 cH and 30 cH show the second peaks at 220.23 nm and 219.67 nm, respectively. *Colchicum* 6 cH and 30 cH show the second picks at 217.65 nm and 220.55 nm, respectively. It is evident that *Cannabis* 6 cH shows a blue shift relative to *Cannabis* 30 cH, but in case of *Colchicum* the 6 cH potency shows a red shift relative to *Colchicum* 30 cH. In both cases the first peak was around 200 nm. The first peak shows much higher intensity than the second one. The MOE values are very low, and for this reason the difference between the spectra is significant. The MOE values are given in the legends to figures.

FT-IR –spectra

The highest frequency of the OH-stretching band is plotted against each test potency in Figure-3. The potencies differ from each other with respect to the peak frequency. While *Cannabis* 30 cH shows the highest frequency, EtOH shows the lowest. The peak frequency of the OH-bending band (v_2) is plotted against each test potency in Figure-4. Here *Cannabis* 6 cH shows the lowest frequency and others differ from each other slightly.

The ratio values of OH-stretching band are all same in all the test potencies and EtOH control (Figure-5). Ratio values of OH-bending band are lowest with *Cannabis* 6 cH, *Colchicum* 6 cH *and Colchicum* 30 cH, but highest with *Cannabis* 30 cH and EtOH (Figure-6). Potencies at 20% EtOH show much higher intensity compared to those at 90% EtOH (Figures-7-10).

DISCUSSION

Ethanol and water form a non-homogeneous solution containing aggregates of water and ethanol, and also free water molecules (Dixit *et al*., 2002; Yoshida *et al*., 2001). It is known that the hydrogen bonding strength is maximum at 20% EtOH (Burikov *et al*.,2010) as used in our experimental study. This phenomenon has been attributed to the difference in hydrogen bonding energy between water-water, water-ethanol and ethanol-ethanol molecules (Dolenko *et al*., 2011). These results concerning the difference in hydrogen bonding energy indicates the occurrence of clathrate hydrate structures in 20% EtOH (Dolenko *et al*., 2011).

UV-spectra

The absorbance intensities of EtOH and water usually occur around 204 nm and 191 nm wavelength, respectively. Peak 1 at lower wavelength region indicates absorbance by water rich clathrate hydrates. The peak II at higher wavelength region represents charge transfer interaction (CT). When oxygen is dissolved by bubbling in some

organic solvents, oxygen serves as electron accepter and organic solvent as electron donor (Tsubomura and Mulleken, 1960). Homeopathic potencies are prepared by successive dilution followed by mechanical agitation or succession in aqueous EtOH. During succession oxygen is dissolved in the solution. This dissolved oxygen participates in CT interaction. In this case oxygen serves as electron acceptor and water or EtOH as electron donor. CT absorbance peak at higher wavelength in UV-spectra has also been reported by others (Ghosh *et al.,* 2008; Tamer 2017; He *et al.,* 2009.) So, CT appears to be an important factor common to all homeopathic potencies.

FT-IR –spectra

Zupancic & Gradadolink (2021) analysed FT-IR spectra of EtOH-water solution and found that EtOH molecules induce changes in the hydrogen bond network of water. These changes depend on the shape and size of hydrophobic unit of the solute (EtOH). Here water serves as a solvent and EtOH as solute. In our study drugs are used as solutes and aqueous EtOH as solvent. These drugs could induce changes in the hydrogen bond network of the solvent. In high dilutions or potencies these induced changes in the network of hydrogen bond may be retained. Water molecules interact simultaneously with the hydroxyl and hydrogen atoms of the methyl group (CH_3) in the ethanol-water solution (Oliveira and vasconcellos, 2006).

In case of the stretching band difference in frequency shift in EtOH control and test potencies may be due to the quantities of the clathrate hydrtae crysrtal and non-hydrogen bonded water molecules (Figure-3). Ratio values of the test potencies and the EtOH control are same indicating similarity in free water molecules and bound molecules (Burikov *et al.,*2010). Mizuno et al (1995) reported that EtOH-water solution shows blue shift with the increase in EtOH concentration. In our experimental study the concentration of EtOH has been fixed at 20%, but there is some variation in the frequency shift of the v_2 band (Figure-4).This variation can be attributed to hydrogen bond strength in water hydrogen in the test potencies and the control. Addition of water to potencies at 90% EtOH increases the free water molecules by 34% to 67% at 3550 cm^{-1} (Figures-7-10). It is evident that free water molecules vary in amount not due to the quantity of water added but due to the quality of the drugs from which potencies have been prepared. The quantity of water added to potencies at 90% EtOH was same for all the test potencies.

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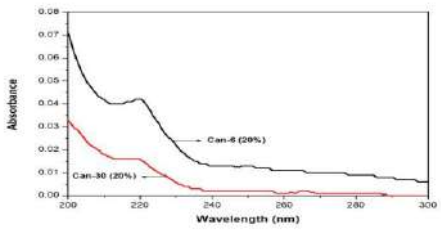

Figure-1: Electronic spectra of the two potencies of *Cannabis sativa* (Can) in 20% EtOH. Each spectrum represents average of 5 spectra, Base line with 20% EtOH. Margin of error (MOE) between Can-6 and Can-30 0.00013%. Since the MOE is very low the difference between the paired spectra is significant.

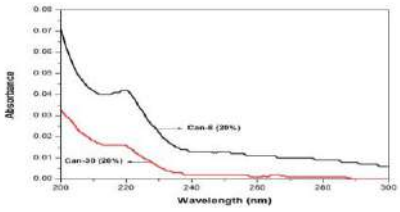

Figure-2: Electronic spectra of the two potencies of *Colchicum autumnale* (Col) in 20% EtOH. Each spectrum represents average of 5 spectra, Base line with 20% EtOH. Margin of error (MOE) between Col-6 and Col-30 0.0006%. Since the MOE is very low the difference between the paired spectra is significant.

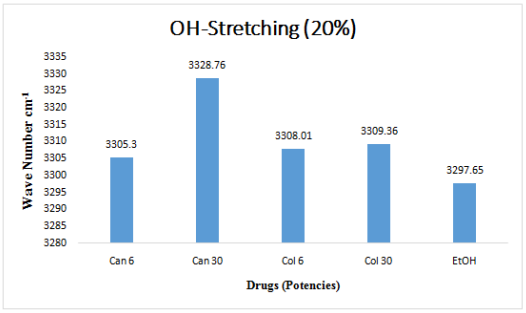

Figure-3: Histogram showing frequency shift of O-H stretching band in EtOH and different potencies 6 and 30cH of Can and Col, all in 20% EtOH.

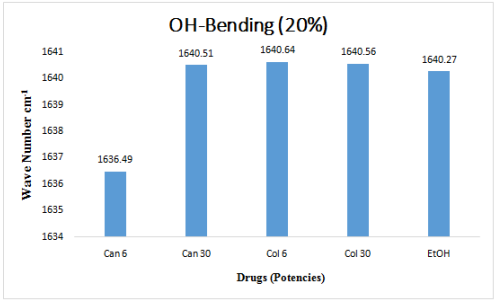

Figure-4: Histogram showing frequency shift of O-H bending band in EtOH and different potencies 6 and 30cH of Can and Col, all in 20% EtOH.

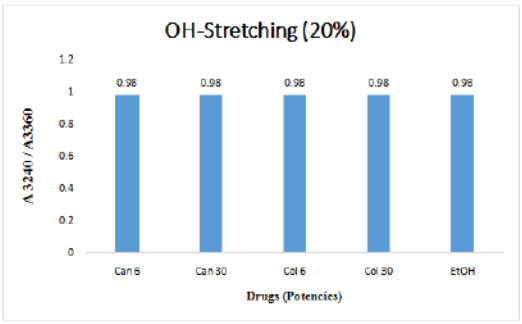

Figure-5: Ratio of absorbance intensities of O-H stretching band of two potencies 6 and 30cH of Can and Col in 20% EtOH at wave number 3240 cm^{-1} and 3360cm^{-1}. 20% blank EtOH also included. Calculation was done after normalization.

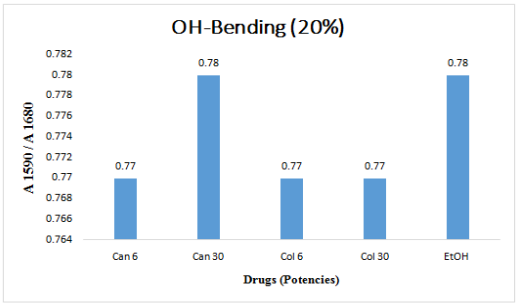

Figure-6: Ratio of absorbance intensities of O-H bending (v_2) band of two potencies 6 and 30cH of Can and Col in 20%EtOH at wave number 1590 cm^{-1} and 1680cm^{-1}. 20% blank EtOH also included. Calculation was done after normalization.

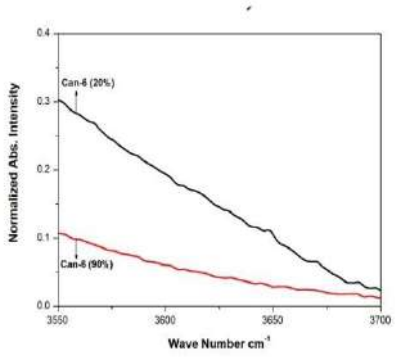

Figure-7: FTIR spectra of the 6th potency showing higher absorbance intensity of 20% EtOH over 90% EtOH by 63%.

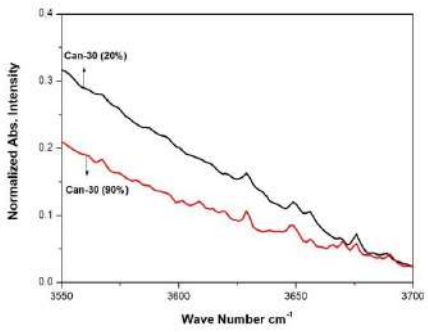

Figure-8: FTIR spectra of the 30th potency showing higher absorbance intensity of 20% EtOH over 90% EtOH by 34%.

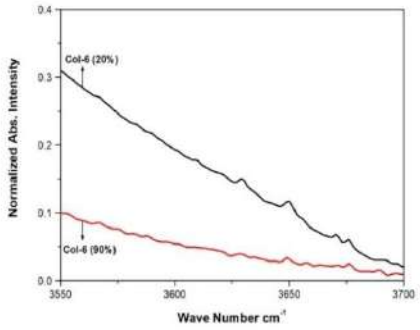

Figure-9: FTIR spectra of the 6th potency showing higher absorbance intensity of 20% EtOH over 90% EtOH by 67%.

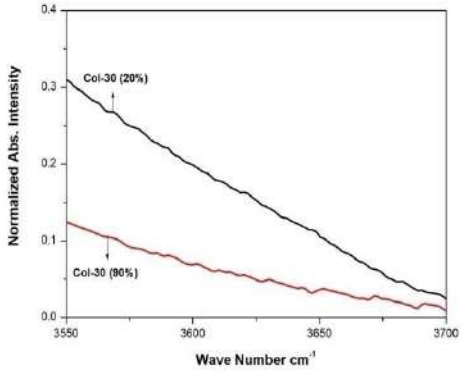

Figure-10: FTIR spectra of the 30th potency showing higher absorbance intensity of 20% EtOH over 90% EtOH by 61%.

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RESEARCH ARTICLE

A Pilot Study to Assess the Effectiveness of Educational Interventional Programme on Disaster Preparedness and Management among Residents in Disaster Prone Areas

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ABSTRACT

A Quasi-experimental study, pre and post-test with control group with quantitative approach was undertaken to assess the effectiveness of educational intervention program on disaster preparedness and disaster management among the residents in disaster prone areas in Kattumannarkoil, Cuddalore. 50 residents were selected by using multi stage sampling technique and data were collected by using Semi-structured Interview schedule and closed ended questionnaire and Observational checklist. Findings reveals that highest percentage (58%) of residents were in the age group of 34-41 years in experimental group and (62%) of them 26-33 years in control group, more or less similar (54% & 56%) percentage of the resident were female in control and experimental group. More or less similar (92% & 90%) percentage of the resident were Hindu religion in control and experimental group. 62% of the residents had High school education in experimental group and 46% of them higher secondary education in control group. Highest percentage (58%) of residents were daily wagers in control group and 44% of residents were daily wagers.60% of the residents had family monthly income Rs.10001-15000 in experimental group and 48% of them had below 10000 rupees in control group, majority 98% and 96% of resident marital status were married in control and experimental group. 72% of the resident belongs to nuclear family in experimental group and 48% of them belong to joint family in control group. More or less similar (78% & 80%) percentage of the resident had previous experience of flood and cyclone disaster in experimental and control group. Percentage wise distribution to assess the level of knowledge regarding disaster preparedness and management among residents before and after EIP which shows that highest percentage (48%) of the residents had good knowledge in experimental group post test and similar lowest percentage (12%) of them had good knowledge in control group post-test & experimental pre-test.

More or less similar highest percentage (36% & 32%) of the residents had average knowledge in post-test experimental group and post-test control group. Percentage wise distribution to assess the level of skill regarding disaster survival kit among residents before and after EIP which shows that highest percentage (88%) of the residents had good skill in experimental group post test. More or less similar highest percentage (48 %, 56% & 52%) of them had average skill in control group pre and post-test & experimental group pre-test. In the present pilot study it can be concluded that educational intervention program on disaster preparedness and disaster management among the residents had Good knowledge and good skill.

Keywords: Knowledge and skill, Disaster Preparedness and Management, Educational Intervention Programme (EIP).

INTRODUCTION

A disaster is a serious disruption to the functioning of rural and urban areas that exceed its capacity to cope using its own resources. Disasters can be caused by natural, man-made and technological hazards, as well as various factors that influence the exposure and vulnerability to a group of people living in a particular geographical area. Disaster preparedness is a set of measures undertaken in advance by governments, nongovernmental organizations, youth clubs, communities, or individuals to better respond and cope with the immediate aftermath of a disaster, whether it is human-induced or caused by natural hazards. The main objective is to reduce the loss of life and livelihoods. Simple initiatives can go a long way, for instance in training for search and rescue, establishing early warning systems, developing contingency plans, or stockpiling equipment and supplies. Disaster preparedness plays an important role in building the resilience of communities. Disaster management is a process of effectively preparing for and responding to disasters. It involves strategically organizing resources to lessen the harm that disasters cause. It also involves a systematic approach to managing the responsibilities of disaster prevention, preparedness, response, and recovery.

Statement of the Problem

A pilot study to assess the effectiveness of educational intervention program on disaster preparedness and disaster management among the residents in disaster prone areas in rural Cuddalore

OBJECTIVES

- To assess the knowledge of disaster preparedness and disaster management before administration of educational intervention program among the residents in experimental and control group.
- To assess the knowledge of disaster preparedness and disaster management after administration of educational intervention program among the residents in experimental and control group.
- To compare the effectiveness of educational intervention program regarding disaster preparedness and disaster management with demographic variables among the residents in experimental group.
- To find out the association between pre test knowledge score on educational intervention program regarding Disaster Preparedness and Disaster Management among the residents with demographic variables in experimental group and control group.
- To find out the association between post test knowledge score on educational intervention program regarding Disaster Preparedness and Disaster Management among the residents with demographic variables in experimental group and control group.

Research Design and Approach

A Quasi-experimental study, pre and post-test with control group with quantitative approach.

Study Setting
The study was conducted in Mamangalam Village and Kunjamedu village, Kattumannarkoil taluk, Cuddalore district.

Population
The target population selected for the study is a resident who is living in the disaster prone area within the age group of 18 – 49 years.

Sampling
The study samples were residents living in Mamangalam Village and Kunjamedu village, Kattumannarkoil taluk, Cuddalore district who fulfilled the inclusive criteria.

Sampling Technique
Multi stage sampling technique was used as a sampling technique for the present study.

Sampling Size
50 residents living in Mamangalam Village and Kunjamedu village (25 control group and 25 experimental group) kattumannarkoil taluk, Cuddalore.

Tool used
Semi-structured Interview schedule closed ended questionnaire and Observational checklist was used to collect the data regarding the effectiveness of educational intervention programme on disaster preparedness and disaster management among residents.

RESULT AND DISCUSSION

50 residents were selected by multi stage sampling technique and data were collected by using Semi-structured Interview schedule and closed ended questionnaire and Observational checklist. The collected data was analysis by inferential statistics. Demographic characteristics reveals that highest percentage (58%) of residents were in the age group of 34-41 years in experimental group and (62%) of them 26-33 years in control group, more or less similar (54% & 56%) percentage of the resident were female in control and experimental group. More or less similar (92% & 90%) percentage of the resident were Hindu religion in control and experimental group. 62% of the residents had High school education in experimental group and 46% of them higher secondary education in control group. Highest percentage (58%) of residents were daily wagers in control group and 44% of residents were daily wagers. 60% of the residents had family monthly income Rs.10001-15000 in experimental group and 48% of them had below 10000 rupees in control group, majority 98% and 96% of resident marital status were married in control and experimental group. 72% of the resident belongs to nuclear family in experimental group and 48% of them belong to joint family in control group.

More or less similar (78% & 80%) percentage of the resident had previous experience of flood and cyclone disaster in experimental and control group. Percentage wise distribution to assess the level of knowledge regarding disaster preparedness and management among residents before and after EIP which shows that highest percentage (48%) of the residents had good knowledge in experimental group post test and similar lowest percentage (12%) of them had good knowledge in control group post-test & experimental pre-test. More or less similar highest percentage (36% & 32%) of the residents had average knowledge in post-test experimental group and post-test control group. Similar lowest percentage (28%) of them had average knowledge in Pre-test experimental group and pre-test control group. Similar highest percentage (52%) of them had poor knowledge in Pre-test experimental group and Post-test control group and lowest percentage (8%) had poor knowledge in Post-test experimental group, Whereas, 44% them had poor knowledge in Pre-test control group.

Similar highest percentage (8%) of them had very poor knowledge in Pre-test experimental group and Post-test control group and lowest percentage (4%) had poor knowledge in pre-test control group. Hence, it can be interpreted that highest percentage of the residents had good knowledge in experimental group it shows that effectiveness of educational intervention programme on disaster preparedness and management among residents. Percentage wise distribution to assess the level of skill regarding disaster survival kit among residents before and after EIP which shows that highest percentage (88%) of the residents had good skill in experimental group post test. More or less similar highest percentage (48 %, 56% & 52%) of them had average skill in control group pre and post-test & experimental group pre-test. More or less similar highest percentage (52 %, 44% and 48%) of them had poor skill in control group pre and post-test & experimental pre-test. Hence, it can be interpreted that highest percentage of the residents had good skill in experimental group it shows that effectiveness of educational intervention programme on disaster survival kit among residents.

CONCLUSION

In the present pilot study it can be concluded that educational intervention program on disaster preparedness and disaster management among the residents had Good knowledge and good skill. This pilot study revealed that the selected methodology and tools were feasible.

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Table1: Comparison of percentage wise distribution to assess the level of knowledge regarding disaster preparedness and management among residents before and after administration EIP in control group and experimental group

Level of knowledge	Control Group				Experimental Group			
	Pre test		Post test		Pre test		Post test	
	F	%	f	%	F	%	F	%
Excellent	0	0	0	0	0	0	2	8
Good	4	16	3	12	3	12	12	48
Average	7	28	8	32	7	28	9	36
Poor	11	44	13	52	13	52	2	8
Very Poor	1	4	2	8	2	8	0	0
Overall	25	100	25	100	25	100	25	100

Table 2: Comparison of percentage wise distribution to assess the level of skill regarding disaster survival kit among residents before and after administration EIP in control group and experimental group

Level of skill	Control Group				Experimental Group			
	Pre test		Post test		Pre test		Post test	
	F	%	f	%	F	%	F	%
Good skill	0	0	0	0	0	0	22	88
Average skill	12	48	14	56	13	52	3	12
Poor skill	13	52	11	44	12	48	0	0
Overall	25	100	25	100	25	100	25	100

RESEARCH ARTICLE

Outcome Prediction for One Day International Cricket Matches

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ABSTRACT

Sports analytics is nowhere an exception in the field of data analytics for they are characterized by a rich and well-recorded database. Cricket serves as one such sport with millions of fans around the globe thereby catering to a vast space for sponsorships and marketing. This paper focuses mainly on predicting the winning team of a One Day International (ODI) cricket match given the competing teams and the location of the match. It also aims in the recommendation of the optimal line up of the players to make the best use of the selected players to produce efficient results. Duration prediction is the final module of the proposed system. Many factors are affecting the outcome of a match such as player performance in each match and player performances with different teams. Based on the 11-member squad of both the teams, the player and match dependent characteristics are taken and the winner is predicted. The winner prediction model is developed using the XgBoost algorithm. A good line-up is recommended for both the teams where the input players are clustered initially using the k-means clustering algorithm and a score for each player is calculated, which aids in determining the position of the player in the line-up. Using the runs scored by the team batted first and the batting performance of the second team against that opponent, the maximum run rate achievable by the second team is predicted using XgBoost Regressor. From this, the duration of the match is obtained.

Keywords: Clustering, Cricket, Match duration, Optimal Line-up, Winner Prediction, Regression

INTRODUCTION

The game of Cricket originated in England and gradually became popular across the globe. The sport is played between two teams of 11 players each. The team that secures the most runs will be declared as the winner. Each team will be given the opportunity to either bat or field in each innings. Apart from the 11 players in each team, few more players can come into play as a substitute under certain circumstances. One of the players in each team will act as the captain and the toss winning captain can either choose to bat or bowl. Only 2 batsmen of the batting team are allowed to be on the ground at any instance whereas all the players of the chasing team must be present. All these rules were framed by the International Cricket Council. In an ODI match, each team will have the opportunity to bat for 50 overs where each over constitutes 6 balls. The goal of the batsmen is to score maximum runs whilst not being stumped and not consuming too much of overs to achieve a better run-rate. Each bowler bowls 6 legal deliveries to call it an over. The non-striking batsman at the end of every over becomes the striker of the next over. An innings is regarded as complete if all the batsmen of team A are out or team B has bowled their full quota of overs. Now, team B comes on to bat in the second innings to chase the target set by team A. Team B is accorded as winners if they achieve the target else, team A is declared as the winner. If the team playing second has secured the same runs as the first team and they are out of wickets or balls, then the match is said to be tied. In such scenarios, super-overs are conducted where each team will be allowed to play for another over, and then the winner is decided. The game is usually monitored by 3 umpires to keep the game fair. The first step for a team to have a better probability to win lies in the line-up of the batsmen which is 2 decided by the captain and the team coach. The existing systems have so far proposed only a mathematical model for determining the line-up and have used only the match characteristics to predict the winner. This will however result in inappropriate results as the team composition plays a major role. For this reason, the paper aims to not only predicting the winning team based on player and match features but also recommending an optimal line up for a team. Another novel idea that is implemented is to predict the duration of the match considering the weather conditions too. This is proved to be very useful for the advertisers so that they can fix prime slots in advance as the viewership increases towards the end of the match.

LITERATURE SURVEY

The relative team strength plays a vital role in predicting the outcome of a match [1]. The model was developed using K-Nearest Neighbor (KNN) which yielded a high accuracy when compared to other classifiers. A method to predict the winning percentage of a team using statistical modeling was proposed [2] which yields an accuracy of 70%. Data of new teams that joined recently and the matches delayed due to rain were considered outliers and were not considered for modeling. Soccer analytics [3] was dealt using Machine Learning approaches to unravel player performances. They have proved that an expert's rating is most dependent on the match outcome. An accuracy of 90% was achieved. After using a weighted average method, the aggregate of performance, an accuracy of 53.4% was produced. Decision Trees and Multilayer Perceptron Network [4] have been used to analyze the effect produced due to these varied factors. The player statistics which is equally important as the match statistics weren't considered here. A method for predicting the player performances [5] by classifying the runs and wickets taken in different ranges has been suggested. Different algorithms such as na¨ıve Bayes, decision tree classifier, random forest, and multiclass SVM were employed to modeling the problems. The features that affect players' performance such as weather or the nature of the wicket was not included in their study. This poses a serious threat to the accuracy of the outcome. The work [6] focuses on selecting the best 11 players from a pool of players to help the IPL Franchises who have spent massive amounts. For such selection, the paper proposes a ranking scheme, a random-forest based recursive feature estimation algorithm. The suitability of the team was measured using a fitness function and the Memetic Genetic algorithm has been designed for search and optimization purposes. A model for predicting the final score of the first innings and estimating the outcome of the match in the second innings for the limited-overs cricket match [7] has been proposed. It uses 2 models-Linear Regression and Na¨ıve Bayes for each innings respectively. Using game characteristics like venue, toss, run rate, wickets remaining, etc. the outcome of the match was predicted. A tool has beed designed for prediction of IPL match winners, visualization of historic match data and provides

player profiling using HBase, a non-relational database [8] serving for the scalability of the application. In [9] a two-module framework was proposed for the sport of soccer. The fuzzy Ranking method was employed for selecting top players and the best combination of players was selected using the Fuzzy Inference system which comprises the second phase.

In [10] different models were developed using SVM, Random forest, logistic regression, decision tree to predict the winner of an IPL before it has started. The decision tree has outperformed the boosting algorithm which is quite unlikely to happen. This inconsistency would most likely be due to improper selection of features in the dataset. Considering only the historical factors and team statistics, [11] provides a prediction model for T20 matches based upon multi-layer perceptron with adjustable factor weightage. Variables considered are home ground advantage, past performances, match experience, performance at the specific venue, performance against the specific opposition, experience at the specific venue and current form. A multiple linear regression model [12] was used to predict the outcome of the game and Duckworth-Lewis method was used to update the predicted runs according to the resources remaining at the end of each completed over. The factors [13] that determine the outcome of the games is not only based on the team's ability and talent of the players but also includes the venue of the match, weather and pitch conditions. Investigation of machine learning technologies [14] [15] have been dealt for predicting cricket match results based on historical match data of the IPL.

Proposed System

As shown in Figure 1, the datasets considered are players and match data for the past 5 years. These datasets are pre-processed separately and then later merged to train various models in each of the steps.

Optimal Line-Up Recommendation

Input: 11-member squad of both the teams
Output: Optimal 11-member squad for both teams
Player statistics dataset is used with features such as the number of fours, sixes, strike rate, etc of each
player in the recently held ODI matches. K-Means, a Clustering algorithm is deployed to group the players after which a score is calculated for each of them to rank them. This ranking corresponds to the line-up position.

Prediction of Match Outcome

Input: Optimal line-up of both teams comprising 11 players each and Ground name
Output: Match Winner
The match dataset is merged with the player's dataset after pre-processing. The model is built and trained with machine learning algorithms like Naive Bayes, XgBoost, Random forest and KNN using the pre-processed data. The trained model gives the winner of the match as the outcome.

Prediction of Match Duration

Input: Projected score of the team batting first
Output: Match duration
Based on the chasing team's batting characteristics and the bowling ability of the batted team, match statistics and the target score, the maximum run rate achievable is determined. From which the match duration, the number of overs the match will last can be predicted.

Data extraction and pre-processing

The player's statistics of ODI matches for the past 5 years are considered for model training. These data are extracted from different sources namely, kaggle.com, howstat.com and espncricinfo.com. The pre-processing steps include removal of duplicate tuples, filling in the missing data, making the data types consistent, giving proper and uniform names to the attributes, removal of insignificant attributes, deriving new batting and bowling features. Advanced processing of the data included the steps which are more specific to the cricket outcome prediction domain. The raw data contained the player statistics of each match comprising both the innings which were later merged as player-

specific data based on location and date attributes. From the existing features like the number of balls bowled, the number of balls faced, the number of boundary fours and sixes, etc., various derived attributes for batsmen and bowlers are calculated. The dataset used for the winner prediction model included the match dataset with the statistics of the corresponding players. Advanced processing of the data included steps that were more specific to the cricket outcome prediction domain. The data regarding abandoned matches, cancelled matches, certain matches with super over were disregarded. The merging of the match with player statistics was done in 2 stages. In the first stage, the player dataset was internally converted by merging inning specific player data with the match dataset. Data about 11 players from each team for both the innings are merged with the match dataset based on 'Ground' and 'Match-date' attributes.

Optimal line-up recommendation

Optimal line-up recommendation is the first phase where the 11-member squad from each team is input and the optimal line up for each of the competing teams is obtained as output. The players are first clustered as openers, middle and lower order batsmen and then are ranked within the cluster to obtain the line up. This is done separately for each of the teams.

Clustering

The 11 players of both the teams serve as input to the first phase of the model. The goal of this phase is to identify the players who are best suited to play as openers, middle and lower-order batsmen. Certain characteristics need to be satisfied by the batsmen to play in any of the above-mentioned categories. The characteristics of the opening batsmen are consistency, defense and adaptability to the new ball at the start of the game. Consistency for a batsman is calculated using the equation (10). A middle-order batsman should have the ability to hit a lot of boundaries and sixes (Hard hitter), the ability to finish the match (Finisher) and score runs quickly (Fast scorer). These qualities are quantified using the formulae (7), (8) and (9) respectively. For the lower order batsmen, strike rate (Batting strike rate) and ability to score runs faster (Fast scorer) are alone enough. These qualities are quantified using formulae (6) and (9) respectively. The opponent also acts as an important factor in determining the order of the batsmen.

Derived bowler features:

$$Bowling\ strike\ rate = \frac{Balls\ bowled}{Wickets\ taken} \quad (1)$$

$$Economy = \frac{Total\ number\ of\ runs\ conceded}{Total\ number\ of\ overs\ bowled} \quad (2)$$

$$Wicket\ Taken = \frac{Total\ number\ of\ balls\ bowled}{Total\ number\ of\ wickets\ taken} \quad (3)$$

$$Consistency = \frac{Total\ number\ of\ runs\ conceded}{Total\ number\ of\ overs\ bowled} \quad (4)$$

Derived batsmen features

$$Batting\ average = \frac{Runs\ scored}{Number\ of\ times\ out} \quad (5)$$

$$Batting\ strike\ rate = \frac{Runs\ scored \times 100}{Balls\ faced} \quad (6)$$

$$HardHitter = \frac{4(No.\ of\ fours) + 6(No.\ of\ sixes)}{To\ \ al\ balls\ faced\ by\ the\ player} \quad (7)$$

$$Finisher = \frac{Number\ of\ times\ not\ out}{Total\ number\ of\ played\ innings} \quad (8)$$

$$Fast\ Scorer = \frac{Total\ runs\ scored}{otal\ balls\ faced} \quad (9)$$

$$Consistency = \frac{Total\ runs\ scored}{No.\ of\ innings\ the\ player\ got\ out} \quad (10)$$

Using these attributes (1…10), the players are clustered into three groups using K-means clustering. The player dataset used for the line-up recommendation module is shown in Table 1.

Ranking

The position of soccer players are identified [9] by ranking them based on the score calculated. A similar approach is used in our usecase to get the lineup of batsmen. Once the clusters for specialist batsmen, bowlers, and all-rounders are obtained, a score is calculated for the opener and middle-order batsmen based on the derived features. Each attribute including the newly derived features is assigned different weights in different categories. The score for each player is the product of the sum of the value of all the attributes. There is a clear distinction between the batsmen and bowlers. Thus, considering the opener and middle order cluster the top two ranked openers are chosen and the remaining are evaluated under the middle order criteria. Finally, the bowlers' cluster is ranked he sample batting line-up for Indian Team is as shown in Table 2.

Match outcome prediction

Two different models were built for predicting the outcome of a match. The first model employs K-Nearest Neighbor, a data classification algorithm that attempts to determine which group a data point is in by looking at the data points around it. The KNN is an example of a ”lazy learner” algorithm, that is, no model is built using the training set until a query of the data set is performed. An algorithm, looking at one point on a grid, trying to determine if a point is in group A or B, looks at the groups to which the points near it belong to. The range is arbitrarily determined, but the idea is to take a sample of the data. If the majority of the points are in group A, then it is likely that the data point in question will be A rather than B, and vice versa. The confusion matrix obtained when KNN was used for classification is shown in Table 3 where the labels 0, 1, 2 represent the loss, win, draw respectively. The outcome prediction model is also built using XgBoost, a popular and efficient open-source implementation of the gradient boosted trees algorithm. The library is laser-focused on computational speed and model performance. Boosting is an ensemble technique where new models are added to correct the errors made by existing models. Models are added sequentially until no further improvements can be made. Gradient boosting is a supervised learning algorithm, which attempts to accurately predict a target variable by combining the estimates of a set of simpler, weaker models. As seen in Table 4, XgBoost has the highest accuracy because it learns the errors from the previous iterations.

Duration prediction

The model predicts the run rate of the chasing team. If the predicted run rate is less than the required run rate, the match will last till 50 overs. The impact of all-out is not considered, in which case the match will wind up even before the overs allotted on the particular day of the match, 50 overs being the majority. In order to predict the run rate regression model is built. With the run rate predicted, the number of overs that would be utilized by the second team is calculated using the formula below (11):

$$Number\ of\ overs = \frac{Target}{Predicted\ run\ rate} \quad (11)$$

Regression

Xgboost is an extreme gradient descent boosting algorithm, where it differs from the gradient descent boosting in the cost function optimization. It uses a second-order partial derivative of the loss function. Also, the training phase is less time consuming with respect to other versions of boosting algorithm. The model learns to fit a learning curve or line by ensemble method. The regularisation parameter allows the learning to regularise in the direction of lesser errors. The value of regularisation ranges from 0 to 1. The regression model yielded a mean square error of 0.16 for the predicted run rate with random samples and an MSE value of 1.92 for the duration of match prediction. The predicted and actual run rates are compared in Table 5. The predicted and actual overs are compared as shown in Table 6.

Experimental results

The recommended batting order of the players for team India and Australia match held on Jan 14, 2020, is shown in Table 3 and Table 7 respectively. The outcome, run rate and winner prediction are as shown in Table 8 and Table 9 respectively.

Analysis and findings

The output analysis is done for the three phases and various findings have been observed.

Optimal Lineup recommendation

The optimal lineup recommendation constitutes the first phase. The output is analyzed by giving two different inputs to the model: India vs Australia and India vs New Zealand. Eleven players from both the teams are fed into the model. The optimal lineup of India, when played with Australia, is shown in Table 10. The lineup almost matches with the actual lineup of the Indian team against Australia in the recent ODI match. The player Shikar Dhawan is misplaced in the middle order category instead of the opening batsman category. Other than him, all other bowlers, openers and middle order players have been placed in their respective categories. This exact categorization has been achieved with the help of the clustering algorithm employed earlier to the input. Without this, the players would have been placed erratically if the score calculation alone is considered. This justifies the importance of the clustering algorithm deployed on the input dataset earlier. The player position is then recommended based on the parameters derived and scores calculated using the historical data of the teams considered. Table 11. shows the lineup of the Australian players against Indian players. The model has recommended the top position for the players Finch and Smith which turns out to be the actual lineup as well. All other players are exactly placed in the middle order and batsmen categories respectively.

Table 12 shows the lineup of India against New Zealand. The lineup recommendation for India Vs New Zealand is also found to be matching with the actual lineup of the respective teams competing with each other in the recent matches. The lineup is influenced by many factors, but the opposition factor is the most influencing one. It is because the formation of a team lineup depends on the performance characteristics of each player who are a part of the team against the opponent in consideration. The major finding from the lineup of the Indian team with New Zealand is that the order is not static for all the competing teams for a given team. The model has taken into account the effect of opposition as well. Hence in such cases, the opening batsmen are prone to be fit in the middle order batsmen category. As shown in Table 12, MJ Guptill is placed in the opening category as per the actual squad as well. Similarly, other players are also properly categorized. The model sometimes fails to provide accurate categorization but not an abrupt misclassification like recommending a batsman for a bowler position. To that extent the models recommend an optimum lineup for almost all combinations of teams provided that there is reasonable historic data involving the teams considered.

Outcome Prediction

The training set of outcome prediction model has a mix of tied matches as well as matches where one of the two teams win. The number of matches that resulted in a tie is very less in proportion. Consequently, the model is unable to predict correctly matches whose result is a tie. As seen in Table 13 and Table 14, the match between India and New

Zealand which resulted in a tie was wrongly predicted with India as the winner. Based on the learning acquired from historic data, the model gives predictions. and hence, any wrong predictions are because those matches did not follow the patterns as in the training data. This can be due to one or more players playing better or worse than what they usually do. These can be considered as outliers.

Duration prediction

On using Xgb Regressor, RMSE for prediction of the maximum run rate achievable is around 0.13365 and RMSE for the duration of the match in terms of overs is 1.497029. In most of the predictions, the duration is off by plus or minus 2 overs as shown in Table 15. The RMSE values of the duration prediction phase can be analyzed in two steps. The first step is the run-rate prediction and the second step, overs calculation. So, the main crux relies on predicting the run rate achievable by the chasing team. The reason for the RMSE value in predicting the run rate being 0.13365 can be due to the insufficiency of the data. For example, for the teams who have been in ODI for the recent years alone, may not have enough data supporting their batting or bowling ability. Also, being a sport of uncertainty due to a major factor like rain, the run rate varies largely with the number of overs that are adjusted during the match. Data supporting such cases involving rain is minimal when compared to the normal case. This adds up to the error in predicting an accurate run rate. In situations where the weather conditions are not favorable like wind or rain, the overs are not 50 as in normal ODI matches. They might be reduced to 25 overs to each team or for the chasing team alone the target and overs may be adjusted. The latter is done using the Duckworth–Lewis–Stern (DLS) method which tends to be fair in certain scenarios only. But they provide very little data to our model. In the worst case, the match might also be abandoned. All these factors sum up for the error in the run rate prediction. As overs are calculated directly from the predicted run rate, the difference in a single over contributes to a nominal amount of error. Considering the India-Australia match held on Jan 14, 2020, our model provided the predictions shown in Table 16 for the team playing in the Second Innings of the match.

Limitations

Matches abandoned or cancelled due to factors such as wind or rain are not considered. Certain tied matches with super-overs are also excluded. Counties like UAE, Oman, Canada, USA and Papua New Guinea are excluded from the dataset. The impact of wickets on the duration of the match is not considered. When the player joins in the team for the first time, no information about him would be available in the dataset. Such cases have not been considered.

Conclusion and future work

In this work, we have discussed in detail about the prediction of ODI matches outcome and duration. We have used the K-Means algorithm for clustering players, XGBoost classifiers for predicting the match-winner and XGBoost Regression for predicting the duration of the match. Boosting algorithms like XGBoost Regression and XGBoost Classifier have proved to give the best accuracy when compared to the counterparts like Decision Tree, Naive Bayes, KNN or SVM. This is because it is an ensemble algorithm that learns from its errors and keeps improving in the subsequent iterations. As future work, we can use ball by ball dataset to make the predictions at different checkpoints during the match instead of predicting at the beginning of the match and the start of the second innings alone. This will involve building a resource table for each of the teams that are similar to the one built through the DLS method but modified for every team based on their past performances. Using this, it is possible to predict the score that can be achieved at any point in time during the game given the overs remaining and the number of wickets lost until that point. Also, we should be able to include the impact of new players about whom no data is available by using the statistics of the nearest player which can be obtained by clustering.

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Table 1: Player dataset

Hardhitter	Finisher	Consistent	batting_ strike_rate	Economy _rate	Consistent _bowler	Innings_Player
0	0	4	25	5.5	27.5	A Mishra
0.2	0	7.5	75	4.831461	14.33333333	A Mishra
0	1	0	100	2.8	14	A Mishra
0.571429	0.5	4	57.14286	5.45	54.5	A Mishra
0.307692	0	5	38.46154	5.6	37.33333333	A Mishra
0.30303	0	56	84.84848	0	0	AM Rahane
0.363289	0	44.1	84.32122	0	0	AM Rahane
0.342593	0	33	76.38889	0	0	AM Rahane
0.390935	0	42.71429	84.70255	0	0	AM Rahane
0.857143	1	0	117.8571	0	0	AM Rahane
0.405941	0	28.6	70.79208	0	0	AM Rahane
0.202899	0	10.57143	53.62319	0	0	AM Rahane
0.495356	0	54.33333	100.9288	0	0	AM Rahane
0.393285	0.090909	34.2	82.01439	0	0	AM Rahane
0.311111	0	24.6	68.33333	0	0	AM Rahane
0.350785	0	58.6	76.70157	0	0	AM Rahane
0.337662	0	37.33333	72.72727	0	0	AM Rahane
0	0	2.5	62.5	4.966667	37.25	AR Patel

Table 2:.Batting Line-up for India

Innings Player
R G Sharma
V Kohli
H H Pandya
M K Pandey
S Dhawan
R R Pant
K L Rahul
B Kumar
R A Jadeja
Kuldeep Yadav
Y S Chahal

Table 3: Confusion matrix on using KNN

Actual Category /Predicted Category	0	1	2
0	85	17	0
1	29	28	0
2	0	1	0

Table 4: Accuracy of models

Algorithm	Accuracy
Naïve Bayes	48.4 %
Random Forest	58 %
KNN	70 %
XGBoost	90. 63%

Table5:. Predicted vs Actual run rates

Predicted run rate per over	Actual run rate per over
5.41486	5.43
4.93358	4.93
4.46857	4.48
5.69324	5.67
5.8339	5.8
5.32154	5.33
5.71082	5.7

Table6: Predicted vs Actual overs

Predicted Runs Per over	Predicted Duration	Actual Duration
4.398	45.7026	46.8531
6.906	43.2957	45.7887
5.425	47.9263	50
6.641	29.9654	30.1515

Table 7: Clusters for Australian players

hard-hitter	Finisher	Consistent	Strike Rate	Economy_rate	Consistent_bowler	Innings_Player	Category_kmeans
0	0.25	5.333333	69.56522	6.108434	39	A Zampa	2
0.571429	0.5	9	128.5714	6.578947	62.5	AC Agar	2
0.443787	0	47.72222	84.714	6.557377	0	AJ Finch	1
1.111111	0.5	104	165.0794	0	0	AJ Turner	0
0.508876	0.25	28.16667	100	0	0	AT Carey	0
0.467797	0	50.63636	94.40678	0	0	DA Warner	1
0	0	3	100	5.087719	16.11111	MA Starc	2
0.592593	0.166667	6.8	125.9259	4.808061	26.36842	PJ Cummins	2
0.434783	0	52.15385	98.26087	9	0	SPD Smith	1
0	0	0	0	5.929204	25.76923	KW Richardson	2
0	0	0	0	4.1	41	JR Hazlewood	2

Table 8: Run rate and winner prediction

Predicted Run Rate (per over)	**5.779418**
Predicted Winner	**2**

Table 9: No.of overs prediction

Predicted no. of overs	**44.1221**

Table 10: Optimal Line-up for Australia

Innings Player
A J Finch
S P D Smith
A J Turner
A T Carey
D A Warner
A C Agar
P J Cummins
M A Starc
A Zampa
K W Richardson
J R Hazlewood

Table 11: Recommended batting order for India with New Zealand

Innings Player
H H Pandya
S Dhawan
R G Sharma
M S Dhoni
V Kohli
K M Jadhav
M K Pandey

K L Rahul
B Kumar
Y S Chahal
Kuldeep Yadav

Table 12: Recommended line up of New Zealand against India

Innings Player
M J Guptill
J D S Neesham
T W M Latham
Kane Williamson
L R P L Taylor
H M Nicholls
C de Grandhomme
M J Santner
M J Henry
T A Boult
C J Anderson

Table 13: Outcome Prediction

Host_Country	**Team_1**	**Team_2**	**Predictions**	**Actual_result**	**Toss_Winner**
Zimbabwe	Afghanistan	Zimbabwe	2	2	Zimbabwe
Zimbabwe	Afghanistan	Zimbabwe	1	1	Zimbabwe
Zimbabwe	SouthAfrica	Zimbabwe	1	1	Zimbabwe
Zimbabwe	SouthAfrica	Zimbabwe	1	1	Zimbabwe
Zimbabwe	SouthAfrica	Zimbabwe	1	1	SouthAfrica
WestIndies	Bangladesh	WestIndies	2	2	WestIndies
England	England	India	2	2	India
England	England	India	2	2	India
Zimbabwe	SouthAfrica	Zimbabwe	1	1	Zimbabwe
India	India	WestIndies	1	1	India
India	India	WestIndies	1	1	WestIndies
NewZealand	NewZealand	SouthAfrica	2	2	SouthAfrica
Australia	Australia	SouthAfrica	2	2	Australia
Bangladesh	Bangladesh	Zimbabwe	1	1	Bangladesh

Table 14: Wrong outcome prediction

Host_Country	**Team_1**	**Team_2**	**Predictions**	**Actual_result**	**Toss_Winner**
NewZealand	Kenya	Netherlands	2	1	Kenya
NewZealand	India	NewZealand	1	3	India
NewZealand	India	NewZealand	1	2	India
NewZealand	Australia	NewZealand	1	2	Australia
Zimbabwe	Afghanistan	Zimbabwe	2	1	Afghanistan
England	Bangladesh	India	2	1	India
Scotland	Scotland	Zimbabwe	1	2	Scotland
England	England	WestIndies	2	1	England
Bangladesh	SriLanka	Zimbabwe	1	2	Zimbabwe

India	India	WestIndies	1	2	India
India	Australia	India	1	2	Australia
Ireland	Bangladesh	Ireland	2	1	Ireland
Ireland	Bangladesh	WestIndies	2	1	Bangladesh
England	India	NewZealand	1	2	NewZealand

Table 15: Duration Prediction match

Actual runrate per over	Predicted runrate per over	Predicted Duration	Actual Duration
2.95	3.0381296	50	50
5.67	5.6293902	27.5341	27.3369
5.12	5.0128818	41.6926	40.8203
4.8	4.886962	49.5195	50
4.79	4.8194704	36.1036	36.3257

Table 16: Duration prediction for India vs Australia

Actual Run Rate	Predicted Run Rate	Actual Overs	Predicted Overs
6.06	5.77	48.4	44.1221

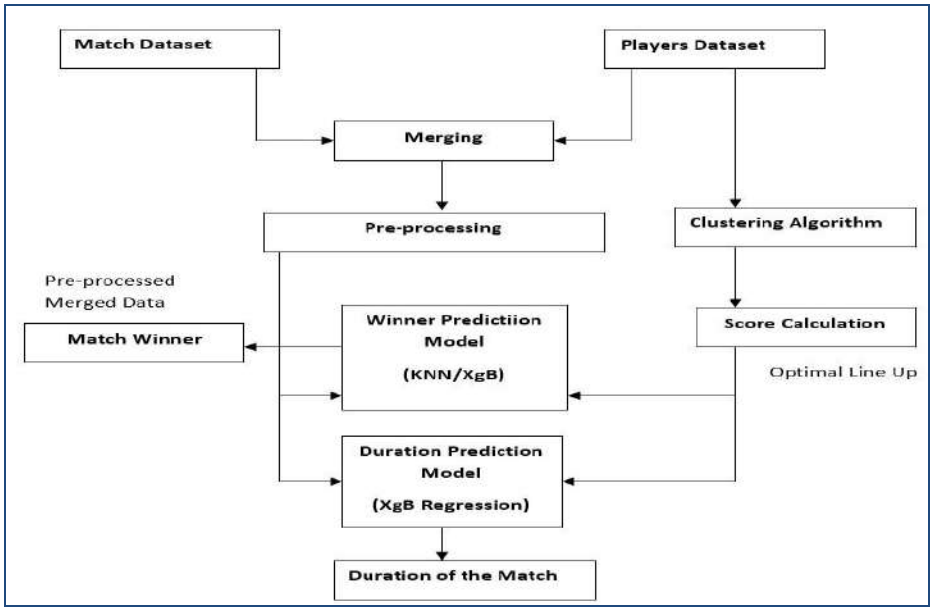

Fig.1 Architecture Diagram

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RESEARCH ARTICLE

Bio Evaluation of Iron Oxide (Fe_3O_4) Nanoparticles using Red Marine Algae *Amphiroa fragilissima* (Linneaus) Jv Lamoroux Aqueous Extract for their Antioxidant Activity

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ABSTRACT

The aim of the present paper is to synthesize iron oxide nano particle using green method. The phytochemicals ,the antioxidant activity of red marine algae *Amphiroa fragilissima*. The phytochemicals present in the selected marine algae *Amphiroa fragilissima* were screened and their antioxidant activity were tested. The marine algae was collected, shade dried, powdered and extracted with aqueous solution .the presence of variety of chemical constituents such as alkaloids, glycosides, phenols, flavonoids, tannins, saponins, carbohydrates, proteins, resins, aminoacids, oils and fats, polysaccharides and terpenoids etc. were analyzed in this marine algae and the formation of iron oxide nano particle by using UV-spectroscopy, FTIR ,XRD their antioxidant activities were studied by DPPH assays. Phytochemicals screening showed the presence of active molecules. The selected algae is having antioxidant potential. From the study. It is clear that these *Amphiroa fragilissima* were the prospective source of bioactive compounds.

Keywords: *Amphiroa fragilissima*, phytochemical analysis, characterization, antioxidant activity

INTRODUCTION

Currently there has been a growing interest in natural antioxidants as replacements of synthetic compounds because of their potential preventive role in several diseases. One possible effective approach for preventing or treating of diseases or disorders is based on diet rich natural antioxidants. Marine macroalgae or seaweeds are one such richest source of natural antioxidants that is recently studied for their potential to decrease the risk of diseases and to improve oxidative stability of food products. Seaweeds are well-known functional food for their richness in several bioactive substances like polysaccharides, proteins, lipids, minerals, certain vitamins and polyphones, with potential medicinal uses against cancer, oxidative stress, inflammation, allergy, diabetes, thrombosis, obesity, lipidemia, hypertensive and other degenerative diseases (El Gamal *et al*., 2010). Moreover seaweed has more than 60 trace elements, minerals, protein, iodine, bromine, vitamins, and several bioactive substances of economic value in providing low-cost, wholesome nutrition and therapeutic protection. In 2011,Gupta and Abu-Ghannamstated that natural antioxidants derived from various plants and marine algae not only have health-promoting benefits, but also have shown a great potential for improving oxidative stability of food products. For the past few decades researchers like Rao *etal*.,1990; Vidyavathi and Sridhar, 1991, Singaravelu *et al*.,2007 were mainly screening on biologically active compounds in different seaweeds against various human pathogenic viruses, bacteria and fungi. They also stated that seaweed is one of the more preferable sources of bioactive compounds which help in preventing oxidative stress and other mammalian diseases as it has more stable antioxidants when compared to terrestrial plants (Rengasamy*et al*., 2019; Kumar *et al*., 2020; Rengasamy*et al*., 2020).

In recent years, novel size-dependent physicochemical properties have led to metallic iron nanoparticles of great potential in a wide range of applications, including magnetic storage media, ferrofluids, biosensors, catalysts (Zhang *etal*.,2005), separation processes, and environmental remediation (Mahdavi *et al*.,2013).However, biosynthesis of metal nanoparticles by seaweed is currently under development. Green nanotechnology makes use of environmental friendly, non-toxic and safe reagents (Salam *et al*., 2012) to reduce or eliminate toxic substances to restore the environment. Thus, the synthesis of nanoparticles has become a matter of great interest in recent times due to their various advantageous properties and applications in a variety of fields. Thus, exploitation of different plant materials and macro algae for the biosynthesis of nanoparticles in green technology is much considered because it does not involve any harmful chemicals, develop unique metabolic systems to survive and leading to the synthesis of a high number of secondary metabolites with potent antioxidant molecules (Rickert *et al*., 2016).Specifically, magnetite (Fe_3O_4) is a common magnetic iron oxide having a cubic inverse spinel structure that exhibits unique electric and magnetic properties based upon the transfer of electrons between Fe^{2+} and Fe^{3+} in octahedral sites (Abhilash*et al*., 2011). Based to their unique physical, chemical, thermal, mechanical properties, and also by having suitable surface characteristics, super paramagnetic nanoparticles it offer a great potential in many biomedical applications, such as cellular therapy, tissue repair, drug delivery, magnetic resonance imaging (MRI), hyperthermia, and magnetofection. Antioxidants are attractive as supplements because of their potential preventive role in several diseases associated with oxidative stress. The antioxidant activity of seaweeds is due to carotenoids, polysaccharides, vitamins, and its precursor and polyphenols, which contribute to the inhibition of oxidation processes (Kumar *et al*., 2019).Such significant seaweed has been screened extensively to isolate lifesaving drugs or biologically active substances all over the world. Still, now, there is scanty information regarding the pharmaceutical potentiality of certain macroalgae species. Thus, the present study was undertaken to identified phytochemical composition in the crude extracts of selected marine macroalgae and to synthesis and characterize iron nanoparticles from aqueous extract of selected marine macro algae with special emphasizes on the antioxidant potential of collected red seaweed from Mandapam to Pamban Coast.

MATERIALS AND METHODS

Selection of sampling site and collection of experimental species

Mandapam in Ramanathapuram district is a small panchayat town, located at 9.28°N 79.12°E at an average elevation of 29 feetis one of the famous tourist attractions with best calm beaches, silent tides, and good water spring and a in South of India. The experimental sample- seaweed was collected from Mandapam beach which falls between the Latitude: 9.2770392 and Longitude:79.1252174 and beneath Pamban bridge with Latitude: 9.2761; Longitude: 79.1867 of Rameswaram East coast of Tamil nadu, India. Live and healthy macroalgae sample was collected by 6.30 a.mduring the lowest tide of chart datum from the seaweed infested locations exclusively from the intertidal rocky and other substratum to avoid other microalgal contamination manually by handpicking method at a depth of 1-2m during the month of October 2021 from Mandapam beach and Pamban bridge of Rameswaram, Ramnad District, Tamil nadu, India.

Transportation and preservation of macroalgae

Manually handpicked seaweed was immediately washed in seawater until all the impurities like the epiphytes, extraneous matter coarse sand and other calcareous impurities were removed. Later they were packed in pre-sterilized polythene bags half filled with seawater and transported to the laboratory with 5 to 6 hrs conditioned under ice at 20ºC to avoid decomposition, loss of metabolites, for identification and future wet lab characterization. After reaching the laboratory they were identified with the help of algologist, Tamil Nadu Agricultural University, (TNAU), Coimbatore, Tamil nadu, India Plate-1.

Preparation of algal powder and crude extract

After identified algal sample was thoroughly sterilized with tap water until undesired impurities, adhering sand particles and extraneous matter such as epiphytes, pebbles, surface salty mature shells were removed. The tap water washed seaweeds were rinsed in the sterile distilled water three times to remove extra sand and dust. Later, the algae were cut into small pieces and scattered over filter paper and left for a few hours to absorb extra water. They algal sample were shade dried for two weeks. Then the samples were turned into coarse powder by grinding them in an electric mixer grinder (Plate.2). The powdered sample was properly packed in ziplock bags and placed in the refrigerator at 4º C. They were then tested for their phytochemicals, nanoparticles and antibacterial activity.

Preparation of Pure Algal Extract (PAE) for wet lab analysis

10 grams of powdered examples were extracted by using soxhelt apparatus and separated with (1:10) solvents like methanol, aqueous, chloroform and ethanol at 40-500c for 8 hrs, and the extraction was filtered by using whatman No 1 filter paper. After extraction was finished, the solvent was then evaporated under vaccum and stored in the refrigerator for further studies. The selected algal species in aqueous extract was used to screen the phytochemical analysis (Ganesan *et al*., 2008) and for the biosynthesis of Fe_3O_4-NPs.

Phytochemical analysis

The phytochemical analysis of aqueous extract from selected algae was screened for the presence or absence of active secondary metabolities such as alkaloids, phenols, flavonoids, anthraquinones, tannins, saponins, coumarins, carbohydrate, proteins, quinines, glycosides and terpenoids. The phytochemicals of the extracts were determined qualitatively as reported by (Janarthanan and Senthil Kumar, 2013) in which powdered seaweed of 10g was soaked in 100ml of water to obtain crude extract. The mixture was then centrifuged at 5000rpm for 20 minutes at 4ºC and filtered using sterilized 0.2µm membrane syringe. A fraction of seaweed aqueous extract of selected marine macroalgae *Amphiroa fragilisima* was subjected to phytochemical analysis. General reaction in these analyses revealed the presence or absence of the compounds in the algal extracts in following procedures.

Biosynthesis of Fe_3O_4 nanoparticles

The magnetite (Fe_3O_4) NPs were prepared using the co-precipitation method described by Kang *et al.* (1996) and Qu *et al*., (1999) with some modifications; the basic reaction is shown below:

$$2Fe^{3+} + Fe^{2+} + 8NH_3 + 4H_2O \longrightarrow Fe_3O_4\,(s) + 8NH^{+}_4$$

The $FeCl_3$ (0.1mol/L) solution was added to the seaweed extract in a 1:1 volume ratio. Fe_3O_4-NPs were immediately obtained with the reduction process. The mixture was stirred for 60min and then allowed to stand at room temperature for another 30 min. The obtained colloidal suspensions were then centrifuged and washed several times with ethanol and then dried at40°C under vacuum to obtain the Fe_3O_4-NPs.

Characterization of biosynthesized Fe_3O_4nanoparticles

Later the aqueous seaweed extract was further exemplified for functional groups and characteristic qualities following characterization techniques given below which was carried out by several processes.

UV-Vis Spectra analysis

The UV-Vis spectrum of Fe_3O_4-NPs was determined using Shimadzu UV-Visible Spectrophotometer (UV-1800). UV-Vis spectral analysis was performed to confirm the biosynthesis of Fe_3O_4-NPs by sampling the aqueous component and the absorption maxima was scanned by UV-Vis spectrophotometer-meter at wavelength of 350–800 nm on Perkin-Elmer Lambda 25 spectrophotometer.

FTIR analysis was used to study the presence of the biomolecules which are responsible for the synthesis of Fe_3O_4-NPs. The biosynthesized Fe_3O_4NPs colloid was centrifuged at 10000 rpm for 15 min and the lyophilized samples were grinded with KBr pellets used for FTIR measurements. The spectrum was recorded in the range of 500– 4000 cm–1 using Thermo Nicolet Nexus 670 spectrometer in the diffuse reflectance mode operating at resolution of 4 cm. Spectral absorption bands were identified in relation to published information.

XRD Analysis

This analysis was done to know the crystal structure as well as size of iron oxide nanoparticle produced. 1ml of the nanoparticle solutions were spread on a glass slide. The slide was dried at 40°c in an oven for 3-4 times to obtain a thin film. Then the spectra were recorded in a philipsxpert pro diffractometer (Cu Kα radiation, λ1= 1.54056; λ2= 1.54439) running at 40 Kv and 30Ma. The 2θ values ranging from 0-100. The crystalline size was also calculated from the half width – maximum height of the diffraction peak of XRD pattern using the Debye-Scherrer equation:

$$D = \frac{K\lambda}{\beta \cos\theta}$$

Where,

D = crystalline size, Ấ
K= crystalline – shape factor
λ =X- ray wavelength
θ = observed peak angle, degree
β = X-ray diffraction broadening, radian

DPPH Radical scavenging activity Principle

The DPPH assay is popular in natural product antioxidant studies. One of the reasons is that this method is simple and sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant. It measures compounds that are radical scavengers. Fig. 1 below, shows the mechanism by which DPPH accepts hydrogen from an antioxidant. DPPH is one of the few stable and commercially available organic nitrogen radicals (Fig.1). The antioxidant effect is

proportional to the disappearance of DPPH in test samples. Monitoring DPPH with a UV spectrometer has become the most commonly used method because of its simplicity and accuracy. DPPH shows a strong absorption maximum at 517 nm (purple). The color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. This reaction is stoichiometric with respect to the number of hydrogen atoms absorbed. Therefore, the antioxidant effect can be easily evaluated by following the decrease of UV absorption at 517 nm.

Procedure

Briefly, prepare 0.1 mM of DPPH solution in methanol and add 100 µl of this solution to 300 µl of the solution of sample Fe2o3 NPs at different concentration (500, 250, 100, 50 and 10 µg /mL). The mixtures have to be shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the absorbance has to be measured at 517 nm using a UV-VIS spectrophotometer. (Ascorbic acid can be used as the reference). Lower absorbance values of reaction mixture indicate higher free radical scavenging activity. The capability of scavenging the DPPH radical can be calculated by using the following formula. DPPH scavenging effect (% inhibition) = [(absorbance of control-absorbance of reaction mixture)/absorbance of control] X 100.

Statistical Analysis

Test samples were carried out independently in triplicates, data was expressed as the Mean± Standard Deviation (SD) and the results were processed using SPPS Ver.19.

RESULTS AND DISCUSSION

Preliminary phytochemical screening is a part of chemical evaluation. The qualitative tests are used to identify the constituents. The quantitative tests are used to quantify or determine the amount of active constituents present. In the preliminary phytochemical screening, sixteen different phytochemicals were analyzed from the aqueous algal seaweed extract of *Amphiroa fragilisima*. Out of Sixteen tests fourteen tests gave positive results and the remaining two tests gave negative results (Table:1). Aqueous extract of *Amphiroa fragilisima* showed the presence of fourteen bioactive compounds like did not show any positive results for their presence in aqueous extract of *Amphiroa fragilisima*. In general, the seaweeds known as therapeutic are rich in secondary metabolites which include alkaloids, glycosides, flavonoids, saponins related active metabolites, which are of great healing value and have been broadly used in the drug and pharmaceutical industry (Eluvakkal *et al*., 2010). The present study observations were directly coincided with the previous observation made by algologist. In conclusion, the results of this phytochemical investigation suggest that the marine red alga *Amphiroa fragilisima* contains important phytochemicals like phenols, flavonoids and fatty acids, which may contribute to its biological activities. The pharmacological properties require further investigation of these active ingredients by implementing techniques of extraction, purification, separation, compound isolation and identification. So, in the present study *Amphiroa fragilisima*is further analysed by synthesizing nanoparticles from the aqueous extract solution and characterized. For the synthesis of *Amphiroa fragilisima* /Fe_3O_4-NPs, firstly, a solution of Fe^{3+} and Fe^{2+} with a 2:1 M ratio was added into the seaweed extract to obtain a yellowish colloidal solution. Then, the freshly prepared 1.0 M of NaOH was added drop-wise to the solution under continuous stirring. The pH of the solution was adjusted to pH 11. The solution was then stirred for 1 h to homogenize the solution and also for the completion of reaction. After that, the as-synthesized Fe_3O_4-NPs were separated by using a permanent magnet. The Fe_3O_4NPs were washed for several times by using deionized water.

The nanoparticles were dried in an oven at around 70 °C for 24 h. The dried sample was stored in an air-tight container for further characterization. All the experiments were conducted at ambient temperature. After addition of NaOH and stirring the solution for 1 h, the color of the reaction mixture of iron chloride salts and seaweed extract changed from light brown to black which indicates the formation of Fe_3O_4-NPs. Separation of Fe_3O_4-NPs could be done with the aid of an external permanent magnet. Plate.3 clearly reveals that the synthesized Fe_3O_4-NPs are able to be attracted by an external permanent magnet quickly, which proved that the nanoparticles possessed magnetic

properties. Once the magnet was removed, the nanoparticles were dispersed readily by shaking. The precipitation occurs because of the Fe_3O_4-NPs have a high tendency to aggregate into agglomerates as to decrease the energy associated with the large surface area to volume ratio, a phenomenon which is likely deteriorated by the low surface charge (Valentin *et al*., 2014).The aqueous extract of *Amphiora fragilisima* was examined under UV visible spectral analysis. The extract was centrifuged at 3000rpm for 10 min and filtered through what man No.1 filter paper for UV spectrophotometer analysis by using high vacuum pump. The sample was diluted to 1:10 with the same solvent. A spectrum was recorded using UV Visible spectrophotometer (Shimadzu UV-2450, Japan) at the wavelength ranging between 200-1100 nm. The characterization of the synthesized Fe_3O_4 magnetic nanoparticles (MNP) by UV– vis spectroscopy is shown in Fig.1. UV–Vis Spectrometry has revealed the characteristic formation of nanoparticles during color change based on the absorption spectra. A scanning wavelength measurement from 200 to 1100 nm was executed to reveal a peak value at 398.75 nm and 445.85 nm which indicated the formation of nanoparticles at absorbance of 0.799 and 0.860 respectively in fig: 2 A characteristic peak at 398.75 nm and 445.85 nm confirmed the formation of Fe_3O_4 nanoparticles. The profile displayed the compounds separated at the nm of 398.75 and 445.85 with the absorption 0.799 and 0.860 respectively which due to the excitation of surface Plasmon vibrations in Fe_3O_4NPs as has been reported earlier by (El -kasass Y., 2016; Rufus *et al*., 2016; Subhashini *et al*., 2018). Effect of precursor salt solution on nanoparticles synthesis revealed that 5mM concentration of Fe_3O_4 resulted in maximum nanoparticles synthesis with the absorption peaks values around 398nm and 445nm Therefore, the selected algae are very efficient in biosynthesis of Fe_3O_4NPs. FT-IR spectroscopy measurements were performed to recognize the possible biomolecules found in *Amphiora fragilissima* aqueous extract which are responsible for reduction and capping of Fe_3O_4-NPs. FTIR is ascribed to functional groups (=C–H, C=O, N– O, C–O, C–N) present in the compound. FTIR spectroscopic studies confirm the presence of amides, phenols, nitrogen, and aromatic compounds that has a strong binding affinity with Fe and thus play a significant role in reducing and capping ferrous ions. The FTIR spectrum reveals characteristic peaks of *Amphiora fragilissima* at 3413.73(cm-1) assigned to N-H stretch, 2891.91 (cm^{-1}) assigned to C–H stretching vibrations, 2969.08(cm^{-1}) assigned to C-H stretch, 1748.39(cm^{-1}) s due to the C–O stretching and N–O asymmetric stretching of the ester group, 1631.11(cm^{-1}) assigned to C=C group, 1415.15 (cm^{-1}) assigned to C=C group, 1107.53(cm^{-1}) assigned to C-O group, 552.45(cm^{-1}) assigned to C-Cl group, and47.172 (cm^{-1}) assigned to Fe-O stretch . It is related to alcohol, phenol group, carboxylic acids, alkenes, alkynes and amines. The present research results corroborate with the FT-IR analysis (Rao, 1963; Bellamy, 1975; Socrates, 1994; Li *et al*., 2004; Kushwaha*et al*.,2013; Thirunavukkarasu *et al*., 2013).

The X-ray diffraction spectrum of the Fe_3O_4 iron nanoparticles is illustrated in (Fig:4). The information obtained from the spectrum indicated that the iron was mainly in its Fe_3O_4 state, characterized by basic reflection appearing at 2Ø value of 30.1°and additional peaks at 36.72°and 58.00°. The obtained broad peak 30.1° revealed that the existence of an amorphous phase of iron and represented bcc (body-centered cubic crystal) Fe_3O_4 lattice plane (110) of Fe_3O_4 (Huang *et al*., 2005).The diffraction peaks of synthesized Fe_3O_4-NPs at 2θ values of 30.1°,35.4°, 43.1°, 53.4°, 56.9° and 62.5° which are assigned to the crystal planes of (220), (311), (222), (422), (511) and (440) respectively. The analyzed diffraction peaks were matched well with the standard magnetite XRD patterns with JCPDS file no:19-0629 which declared the crystallographic system of cubic structure. This result corroborates with the results with respect to its reflection peaks positions Wang *etal* (2017). Besides, the synthesized nanoparticles were affirmed to be Fe_3O_4 but not maghemite (γ-Fe_2O_3) by comparing the XRD patterns with the standard maghemite JCPDS file no.: 01-089-3850. A huge difference can be clearly seen in that the XRD pat-terns of γ-Fe2O3consist of many peaks, unlike Fe_3O_4which only involves few peaks. This is confirmed by a study which showed unanimous XRD patterns of sea-weed *K. alvarezii* .In 2010 Wu *et al*., stated that algae generally have higher antioxidant activity due to a higher content of nonenzymatic antioxidant components, such as ascorbic acid, reduced glutathione, phenols and flavonoids. Halliwell, 2007 stated that recently, polyphenolic compounds including flavonoids is known as safe and non-toxic anti-oxidants. Many studies have shown that a high dietary intake of natural phenolics is strongly associated with longer life expectancy, reduced risk of developing some chronic diseases, various types of cancer, diabetes, obesity, improved endothelial function and reduced blood pressure. Saeidnia *et al*.,2012 and Boonchum*et al*., 2011 have reported that the seaweed of all the classes possess antioxidant activity. DPPH reagent has been used extensively for investigating the free radical scavenging activities of compounds. In the DPPH test, the dried extracts are potentially

able to produce the yellow coloured diphenylpicrylhydrazine. The assay is based on the reduction of alcoholic DPPH solution in the presence of hydrogen – donating antioxidant due to the formation of the nonradical form DPPH-H by the reaction. DPPH results are often interpreted as the "Inhibition concentration". Among the various fractions 500 µg/ml, 250 µg/ml 100 µg/ml, 50 µg/ml; 10 µg/ml. Aqueous extract concentration showed maximum biological activity. Among the dosage fractions the percentage inhibition recoded in 10 µg/ml was 61.25%; in 50 µg/ml it was 65.50% while in 100 µg/ml there was an increase in inhibition percentage which was about 67.93%. Further the dosage fraction was increased to 250 µg/ml and upto 500 µg/ml in which a maximum inhibition percentage of 73.90 to 77.91% inhibition was recorded (Fig:5). Thus, it was observed that the percentage of inhibition increases with the concentration of the extract in all samples so the radical scavenging activity is dose-dependent. The difference in the inhibition could be due to the presence of various compounds including pigments (chlorophyll a, b, carotenoids), alkaloids, and phenolic compounds which can participate in the great antioxidant activity. This means that synergistic effects may occur between these constituents leading to the pronounced antioxidant activity of algal extract (Heo and Cha,2005; Shanab*et al*.,2011; Yang *et al*.,2014). The highest free radical scavenging activity was observed in different algae species belonging to different phyla (Moharram and Youssef, 2014; Bianco *et al*., 2015) .

CONCLUSION

The present research study has been proposed with an aim of green approach for synthesizing iron oxide nanoparticles using low-cost marine red seaweed *Amphiroa fragilissima* a reducing mediator and further investigated the nanoparticles formation using UV-Vis spectrophotometer. The possible biomolecules amide and polyphenol groups may responsible for the reduction of Ferric chloride to iron oxide nanoparticles are identified by FT-IR. The formed metal nanoparticle was found to have wider antioxidant activity .iron oxide nano particle plays a vital role in bioremediation, removal of heavy metals in water sources and it can be also in the field of biomedicine for target drug delivery, magnetic resonance imaging, cell separation and detection, tissue repair, magnetic hyperthermia etc.. Thus, the iron oxide nanoparticles have great promising for application in biological based nanomedicine, biosensors and food industries with development of easy, reliable and eco-friendly methods helps in endorsing extra interest in the synthesis and application of nanoparticles which are good for mankind.

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Table:1 Phytochemical screening of aqueous extracted seaweed collected from Mandapam, Rameswaram coast, Tamilnadu

S.No	*Phytochemical*	*Amphiroa fragilisima*
1	Alkaloids	**+++**
2	Tannins	**++**
3	Glycosides	**+**
4	Carbohydrates	**+++**
5	Flavonoids	**++**
6	Proteins	**+++**
7	Resins	**+**
8	Saponins	**++**
9	Anthrocyanin	+
10	Amino acids	**++**
11	Steroids	**-**
12	Terpenoides	**++**
13	Phenol	**++++**
14	Oils and fats	**+**
15	Polysaccharides	**+**
16	Dipterpenes	-

-- = Negative, + = Positive, ++ = Moderate, +++ = Highly Positive

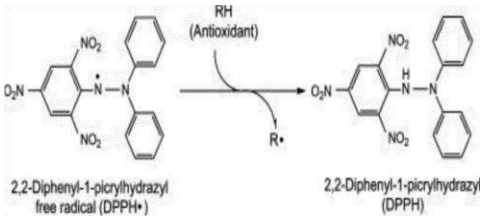

Fig1: Reaction of antioxidant activity

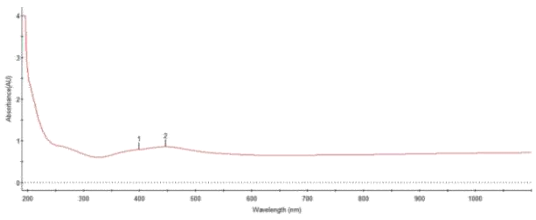

Fig 2 : UV.Vis Spectral analysis of *Amphiora fragilissima* aqueous extract

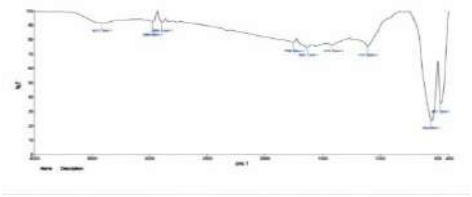

Fig :3 shows FTIR analysis ofFe3O4-NP synthesised from *Amphiora fragilissima* aqueous extract.

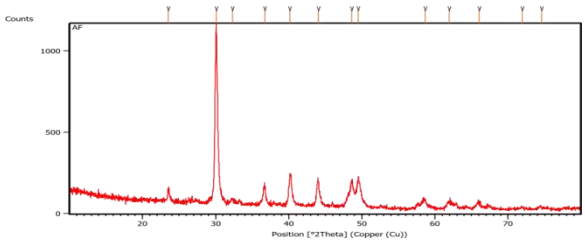

Fig. 4 XRD pattern of Fe3O4-NP synthesised from *Amphiora fragilissima* aqueous extract

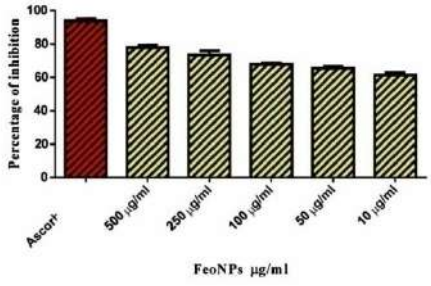

Fig :5 DPPH radical scavenging activity (%) of Fe3O4NPs aqueous extracts of *Amphiroa fragilissima*

Plate -1: Experimental Algae-*Amphiroa fragilissima*

Plate. 2 Algal powders prepared from dried sample

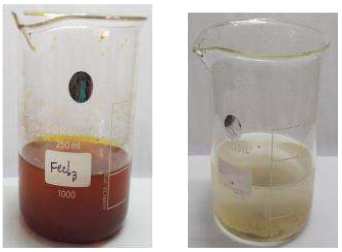

Plate: 3 Synthesized of Fe3O4-NPs

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RESEARCH ARTICLE

Design, Synthesis and Cytotoxic Investigations of New Mixed Ligand Metal Complexes

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ABSTRACT

Mixed ligand complexes based L-tryptophan (Try) have been synthesized and characterized by employing spectrophotometric methods involving Elemental analysis, UV-Visible, FTIR,VSM,SEM and PXRD studies. The complexes were found to have the composition $[Mn(TMBAAP)(Try)(H_2O)_2]$ and $[Cd(TMBAAP)(Try)(H_2O)_2]$. A six coordinated octahedral geometry is proposed for these complexes based on magnetic susceptibility and electronic spectral data evidence. The free ligand and the mixed ligand complexes were tested against MCF-7 breast cancer cell lines. The Manganese and Cadmium complexes showed IC_{50}values of 7.49 and 6.85 which have shown good activity compared to standard drug doxorubicin. The complexes also exhibited significant anti-fungal and anti-bacterial activity towards standard drug fluconazole and Neomycin.

Keywords: L-tryptophan, SEM, P-XRD, MCF-7, Anti-fungal, Anti-microbial activity.

INTRODUCTION

Flyspeck etiquettes in bioinorganic chemistry area unit crucial for enhancing the outline of compounds to minimize toxic side impact and acknowledge their mechanism of action. A strong malignant tumor agent ought to own inherent, repressive property and additionally delivery, indefinite quantity and duration in vivo [1]. Organic operate and conformation of mutated sequence is also altered by approach of binding of metal ions. Upswing of activities relying on the structural information, intending in enhancing and growing totally different styles of metal based mostly compounds, continuous obtain of additional metal based mostly compounds are synthesized via revamping the prevailing chemical form via substance substitution[2]. The prevalent research paper addresses the stylish

development within the style of novel antineoplastic agents based on transition metal complicated via highlight the close to chemical analysis among their structural alternatives and cytotoxic ability[3,4,5].Cancer is brought on once genetic damage to the cells prevents them from listening to regular tissue controls. The foremost cancers spreads once affected cells multiply chop-chop, forming tumors of variable tiers. Special healing procedures may be used, betting on however a protracted approach the foremost cancers have unfold. Malignant neoplasm medicine are classified as therapy, secretion remedy and therapy. Therapy protected variety a families outlined by approach of every their chemical structure and mechanism of action, alkylating agents, antibiotics, antimetabolites, cell division inhibitors [7,8]. Metal complexes play an important role in drug remedy.

Themetallo-elements gift in shred amount performs very important perform in residing device on the molecular stage. Transition metals exhibits varied oxidization states and would possibly interact with variety of charged molecules. This property of transition metal has unraveling the metal primarily based medicine with promising pharmacologic applicability [11]. Mixed ligand domicile are a significant class of co-appointment accumulates and have pulled in extraordinary premium which are gotten from Schiff bases [13]. The combination of an assortment of mixed ligand assortment of only one metal particle is conceivable and by thinking about various particles, countless such compounds can be originated. Mixed ligand complexes have been found to act as an active catalyst in reactions of industrial consequence, hydrogenation, hydroformylation and oxidative hydrolysis of olefins [15,16]. The studies on synthesis, characterization and biological evaluation gained significant importance in emerging field of co-ordination chemistry. During the tenure of this work, we strived preparation, characterization of novel group with Manganese and Cadmium metal ions and also find out the anti-microbial and cytotoxicity against selected human breast cancer cell lines. Thus, we have focused on studying Mn(II) and Cd(II) mixed ligand metal complexes of the general composition $[M(TMBAAP)(Try)(H_2O)_2]$ where (M = Mn, Cd) metal ions, TMBAAP = [4-(3,4,5-Trimethoxy benzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one],Try = L-tryptophan.

MATERIALS AND METHODS

All synthetic compounds were obtained from business sources and used without being cleansed. Elemental analysis (EA) of C,H,N,O were performed using CHNS/O-2400 series II elemental analyzer, IR spectra were recorded on Bruker-FT-IR spectrometer on KBr pellet in the wave number range of 4000-400 cm^{-1}.Electronic spectral studies were conducted on a UV-Visible 1800 series, with wavelength of 200-400 nm. Magnetic susceptibility was determined using Vibrating sample Magnetometer.Model-7410 series VSM, the magnetic moment in the range of 2-40 GHz were detected.SEM images are acquired from scanning electron microscope. FLEX- SEM 1000 instrument, Nano size particles nearly 1-100 nm are detected. Powder XRD patterns were studied using Bruker-D8-Advance powder X-Ray Diffractometer, gives details on crystallite nature and structure elucidation of a compound.

EXPERIMENTAL

Synthesis of Ligand [TMBAAP]

3,4,5-Trimethoxybenzaldehyde (2g) (0.1M) is taken and dissolved in 50 ml of methanol. The 4-Amino antipyrine, (2.2g) (0.1M) is dissolved in 50 ml of distilled water. These solutions were mixed in a clean 250ml round bottom flask and stirred with a magnetic stirrer. This reaction mixture was excited in water bath by refluxing for one hour. On cooling and slow evaporation at room temperature, yellow colored precipitate was formed. The solid was separated by filtration and washed several times with hot water .Then it was dried in vacuum. The compound was recrystallized from methanol. The percentage of yield is 85%. The formation of Schiff base is shown in [Scheme -1].

Synthesis of Mixed ligand metal complexes $[M(TMBAAP)(Try)(H_2O)_2]$

To the warm methanolic solution of 10 ml of 4-(3,4,5-Trimethoxy benzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one[TMBAAP] (0.1M) as a primary ligand was added to 10 ml of warm methanolic solution of metal chloride(Mn, Cd) (0.85g)(0.05M). After 30 min, 5ml of methanolic solution of L-Tryptophan (2.2g) (0.1M) amino acid was added as Co-ligand, the mixture was stirred vigorously. Further, the mixture was refluxed for about 2

hours. The resultant precipitate were altered from yellow to pink and orange colored crystals. The obtained crystals were filtered washed with warm water and dried in vacuum. The percentage of yield was 76%. The formation of mixed ligand metal complexes was given in [Scheme-2].

RESULTS AND DISCUSSIONS

FT-IR Spectral studies

In the IR spectra of Mn(II) complex [Mn(TMBAAP)(Try)(H_2O)$_2$] it can be seen that υ(C=N) stretching vibrational frequencies appeared in the region 1550 – 1594 cm^{-1}. The corresponding band frequencies in the free ligand were observed in the region1630cm^{-1}. The bands due to aromatic υ(C=C) stretching frequency were observed in the region 1550 cm^{-1} as in the free ligand observed at 1580 cm^{-1}. The absence of ligand bands in the region 3418 cm^{-1} due to hydrogen bonded enolic O-N-H stretching frequency in Mn(II) complex confirms that the enolic carbonyl oxygen coordinates with metal in the region at 3407 cm^{-1}. The new bands in the region 546 -419 cm^{-1} in the spectra of Mn(II)complex indicate the vibration of (M-N) and (M-O)bonds. In Cd(II) complex [Cd(TMBAAP)(Try)(H_2O)$_2$]υ(C=N) vibrational frequency bands appeared in the region of 1496 – 1578 cm^{-1} were assigned to υ (C=N) stretching frequency. These are shifted towards lower frequency side than the corresponding free ligand. The lowering in frequency indicated the coordination through nitrogen of azomethine υ(C=N) group in complex**.** The new bands in the region 539-487 cm^{-1} in the spectra of Cd(II) complexes indicate the vibration of (M-N) and (M-O)bonds respectively. The FT-IR spectra's of ligand and complexes are illustrated in [Fig-1, 2, and 3] and FT-IR data were shown in [Table-2].

Electronic Spectral Studies

Manganese (II) ion has d^5-configuration and is capable of forming spin-free as well as spin-paired complexes. It is not possible to observe the d-d spectra of the Mn(II) complexes with organic ligands. Since the weak tail of ligand absorption in to the visible region is often enough to mark the d-d transition bands the electronic spectra of the Mn(II) complex [Mn(TMBAAP)(Try)(H_2O)$_2$] shows one weak band at 23,809 cm^{-1}, which is assigned to (CT) Charge Transfer transition. This transition indicate the octahedral geometrical environment around the Mn(II) ion. The electronic spectrum shows excessive high intense absorption band at 23,809 cm^{-1}which signify (CT) Charge Transfer transition. Electronic transmission spectra of Cadmium complex [Cd(TMBAAP)(Try)(H_2O)$_2$] did not exhibit d-d transitions because its d^{10} orbital is filled but exhibit absorption bands due to ligand to metal charge transfer (LMCT) indicating the d^{10} system. This transition indicate the octahedral geometrical environment around the Cd(II) ion. Physico-chemical, electronic spectral data, magnetic moments of ligand and mixed ligand metal complexes are presented in [Table-1].

Magnetic Susceptibility

The Manganese (II) complex are expected to show magnetic moment close to spin-only value for five unpaired electrons. Mn(II) complexes are generally spin-free complexes. At room temperature the magnetic moments of some of the Mn(II) complexes closely corresponds to the value for three unpaired electrons instead of five unpaired electrons. In present work, the magnetic moment value of mixed ligand complex [Mn(TMBAAP)(Try)(H_2O)$_2$] lies at 5.85 B.M. at room temperature. This value indicates an octahedral geometry as expected (5.92 B.M.) for a high-spin d^5-system. In Cadmium (II)'d' sub shell is completely filled. There are no unpaired electrons, hence the compound of Cd(II) are expected to be diamagnetic. The Cd(II) complex is expected to be octahedral . In present work the magnetic moment determination of [Cd(TMBAAP)(Try)(H_2O)$_2$] complex shows diamagnetic nature.

Scanning Electron Microscope

The SEM micrograph for mixed ligand 4-(3,4,5-Trimethoxy benzylideneamino)-1,2-dihydro-2,3-dimethyl-1-phenylpyrazol-5-one [TMBAAP]along with metal ions Mn(II),Cd(II) are shown in figures. It can be seen that morphology was drastically changed in mixed ligand complex. The SEM micrographs of mixed ligand complexes, signifies the formation of Nano sized particles, they are extremely agglomerated in nature due to induced crystal

growth itself. In SEM images perceptible phase separations in dense layer was observed. The province size of Manganese with 1µm and cadmium with 1µm metal complexes were detected. The Electron dispersive spectra of Mixed ligand complexes [M(TMBAAP)(Try)$(H_2O)_2$] were taken. The sample was coated on the platinum plates at room temperature. The EDAX studies of mixed ligand metal complexes show compositions of the nano structured complexes. The EDAX analysis data matched with C, H and N elemental analysis result. The EDAX spectrum is shown along with SEM images and graphs, which gives details of elemental proportions present in the complexes [Fig 4 – Fig 7].

Powder –XRD studies of metal complexes of [M(TMBAAP)(Try)$(H_2O)_2$]

In the present research work Powder X-ray diffractional studies have been employed to investigate mixed ligand metal complexes. The powder x-ray diffraction spectroscopy was used to check the crystallinity of the synthesized mixed ligand metal complexes. The structures of complexes were confirmed by Powder XRD recorded at (2ɵ) and 20-80^{0}c. All the mixed ligand Mn(II),Cd(II) metal ion complexes show broad and intense peaks. The line broadening of the crystalline diffraction peaks shows higher crystallinity and fine nanoparticles. The experimentally obtained value of d and calculated one along with the relative intensities for the selected peaks were tabulated. The unit cell parameters for the highest peaks and $h^2+k^2+l^2$ values were deduced from the spectra. The values for both Manganese and Cadmium complexes are 1,3,5,6,7,9 and the presence of forbidden number 7 in these values indicated the complex may be tetragonal or hexagonal system and the lattice parameters were calculated as a=b=c=9.34 A^0 and are displayed in [Fig 8 - Fig 11].

Anti-cancer activity

In a previously sterilized 96-well plate a mixture of freshly cultured MCF-cell line and testing samples were added in the presence of minimum essential media (MEM) with 10% inactivated fetal calf serum and the plate was incubated for 48 hrs in the presence of 5% CO_2.After incubation, 100 µL of DMSO was added to dissolve purple colouredforamazon crystals. The optical density of the out coming coloured solutions was recorded at 570 nm in a spectrophotometer. The doxorubin was used as reference drug and DMSO as a negative control. The cytotoxicity is expressed in terms of IC_{50} (µg/mL) which is the lowest concentration of the drug molecule that inhibited proliferation rate of cancerous cells by 50% as compared to the untreated cells. This can be obtained by plotting the percentage of cell viability versus concentration of the drug molecule [Table-3].

Antimicrobial activity

The antimicrobial activity of Schiff base ligand [TMBAAP] and its metal complexes was observed at various concentrations *viz.* 100 (µg/µL), 150 (µg/µL), 200 (µg/µL) and 250 (µg/µL) against two species of bacteria i.e. *S. aureus* and *K. pneumonia*, and two species of fungi i.e. *A. niger* and *Trichophytonrubrum*.

Antibacterial activity

Results have shown the moderate antibacterial activity of mixed ligand manganese complex when compared with standard drug. The range of inhibition for Mn-complex against *S. aureus* was found to be between 7.1mm to 8.1 mm, for *K. pneumonia* it was 7.8 mm to 8.3 mm. Results have shown the moderate antibacterial activity of mixed ligand cadmium complex when compared with standard drug. The range of inhibition for Cd-complex against *S. aureus* was found to be between 5.8 mm to 6.3 mm for *K. pneumonia* it was 5.4 mm to 6.1 mm.

Antifungal activity

Results have shown the moderate antifungal activity of mixed ligand manganese complex when compared with standard drug. The range of inhibition for Mn-complex against *Aspergillusniger* was found to be between 8.1 mm to 8.9 mm for *Trichophytonrubrum*it was 8.3 mm to8.8 mm. Results have shown the moderate antifungal activity of mixed ligand cadmium complex when compared with standard drug. The range of inhibition for Cd-complex against *A.* niger was found to be between 6.1 mm to 6.7 mm for *Trichophytonrubrum*it was 6.3mm to 7.0 mm.

CONCLUSIONS

The synthesized ligand and mixed ligand metal complexes [Mn, Cd] have been characterized by employing spectroscopic techniques, Elemental analysis, FTIR,UV-Visible, VSM,SEM and PXRD studies confirmed the structure and properties of ligand and metal complexes. The findings of transitions revealed from electronic spectral data and magnetic moments attained suggests that the complexes exhibit octahedral geometry. The mixed ligand complexes have shown enhancing activity against bacterial species and fungal species similar to standard drugs neomycin and fluconazole. The free ligand (TMBAAP) and mixed ligand metal complexes were used to determine the cytotoxic effect against MCF-7 cell line by MTT assay. The Cadmium complex exhibited good anticancer activity with anIC_{50} value equal to 6.85 µg/mL which is very nearer to the standard drug. The free ligand and manganese complex have displayed good to moderate activity, which we hope will aid in the development of new drugs to control cancer activity.

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Table 1:Physico chemical and electronic spectral data of Schiff base and mixed ligand complexes

Compound	Molecular weight(gm/mole)	Colour	(Yield %)	UV data(Assignment)	Magnetic Moments. M_{eff}(B.M)
[TMBAAP]	461.45	Yellow	85	-	-
[Mn(TMBAAP)(Try)(H_2O)]	829.75	Pink	72	Charge transitions	5.85
[Cd(TMBAAP)(Try)(H_2O)$_2$]	874.75	orange	76	Charge transitions	Diamagnetic

Table 2: FTIR data of ligand and mixed ligand metal complexes

Ligand \ Metal complexes	C=O	C=N	C=C	C-N	C-O	M-N	M-O
(TMBAAP)	1698	1598	1553	1355	1295	–	–
[Mn(TMBAAP)(Try)(H_2O)$_2$]	1682	1574	1556	1337	1231	546	419
[Cd(TMBAAP)(Try)(H_2O)$_2$]	1693	1598	1552	1357	1294	485	444

Table 3: Inhibition of cell viability of Mixed ligand metal complexes

Compound	IC_{50}(µg/mL)
[TMBAAP]	11.05
[Mn(TMBAAP)(Try)(H_2O)$_2$]	7.49
[Cd(TMBAAP)(Try)(H_2O)$_2$]	6.85
Doxorubicin	6.02

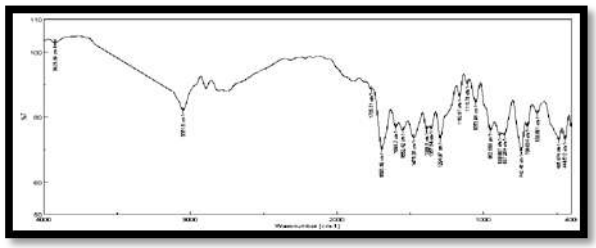


Figure 1: FTIR data of Schiff Base ligand [TMBAAP]

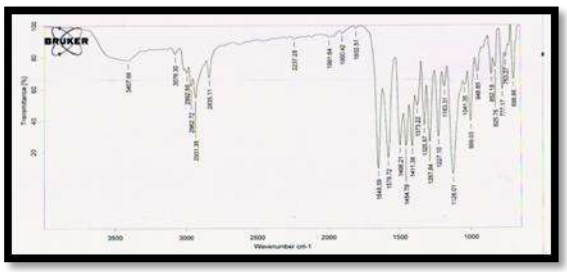


Figure 2: IR Spectra of Mixed ligand Manganese (II) Complex

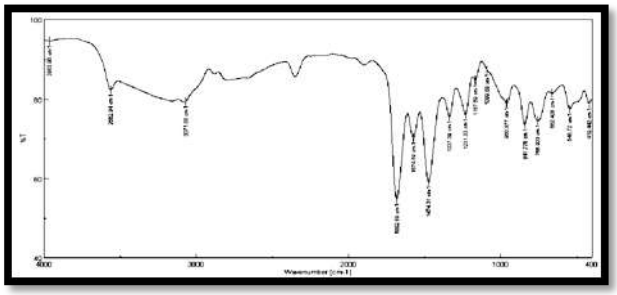


Figure 3: IR Spectra of Mixed ligand Cadmium (II) Complex

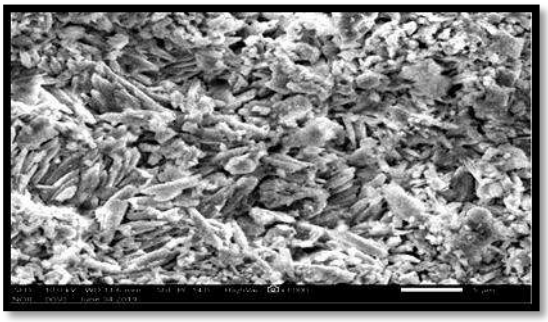


Figure4: SEM image of Mn(II)complex(1nm)

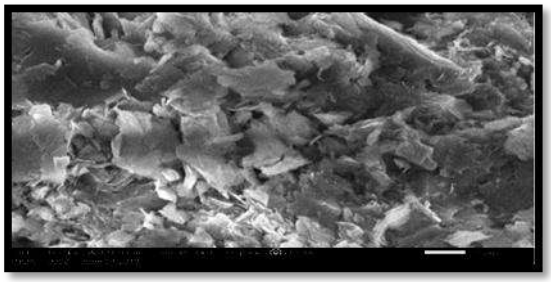


Figure5: SEM image of Cd(II)complex(1nm)

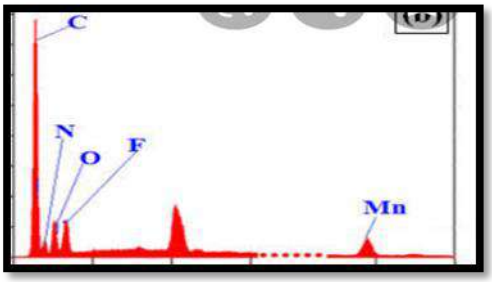


Figure 6:EDAX graph of Mn(II)complex

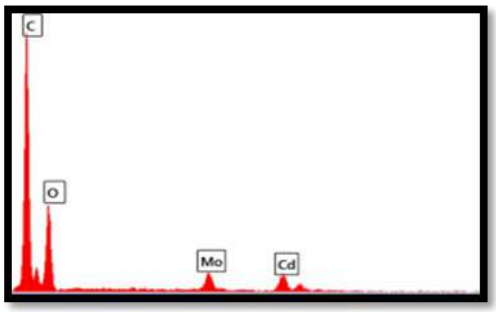


Figure 7:EDAX graph of Cd(II)complex

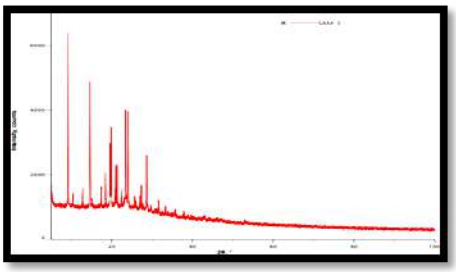


Figure 8: PXRD pattern for Mn(II)complex

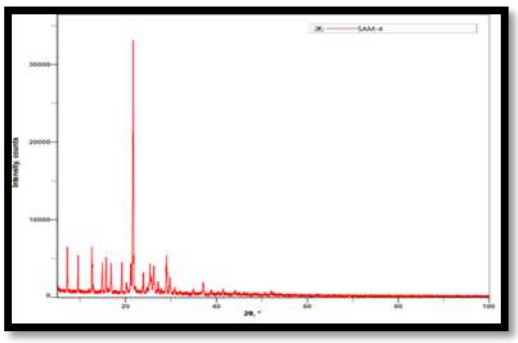


Figure 9:PXRD pattern for Cd(II)complex

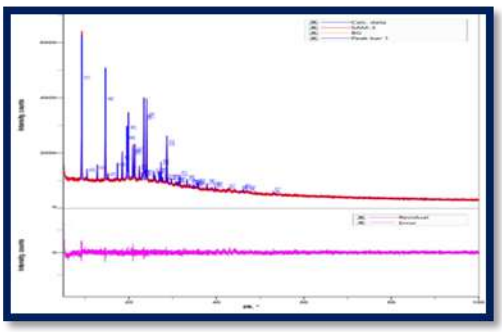


Figure 10:Peak profile for Mn(II)complex

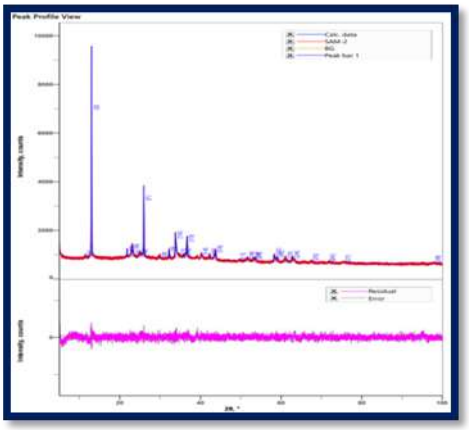


Figure 11:Peak profile for Cd(II)complex

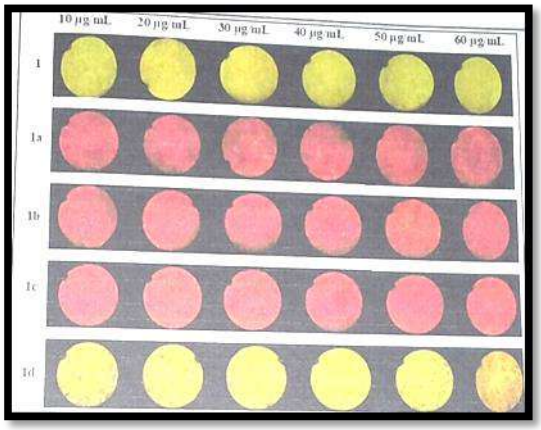


Figure 12: Anti-cancer activity of mixed ligand metal complexes

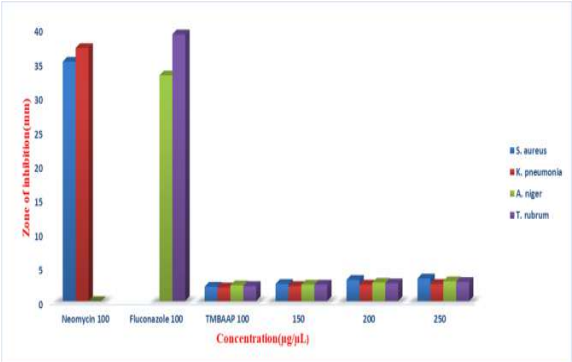


Figure 13: Graphical representation of antimicrobial activity of Schiff base (TMBAAP) in terms of zone of inhibition against bacteria and fungi.

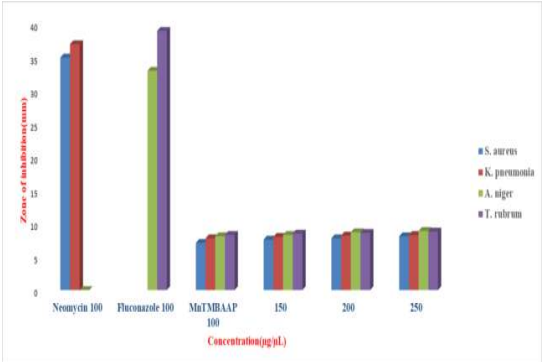


Figure 14: Graphical representation of antimicrobial activity of Mixed ligand Mn(II) Complex in terms of zone of inhibition against bacteria and fungi.

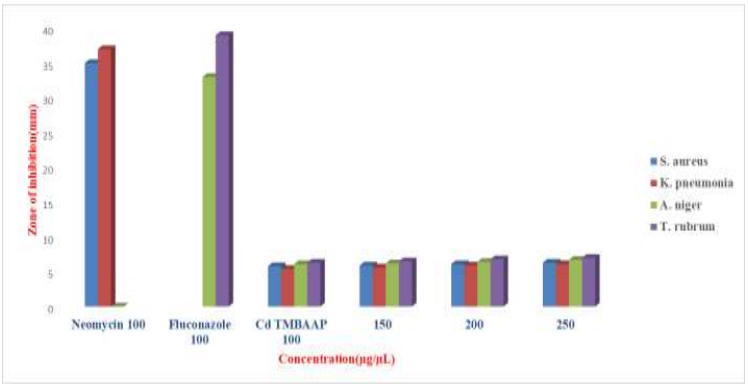
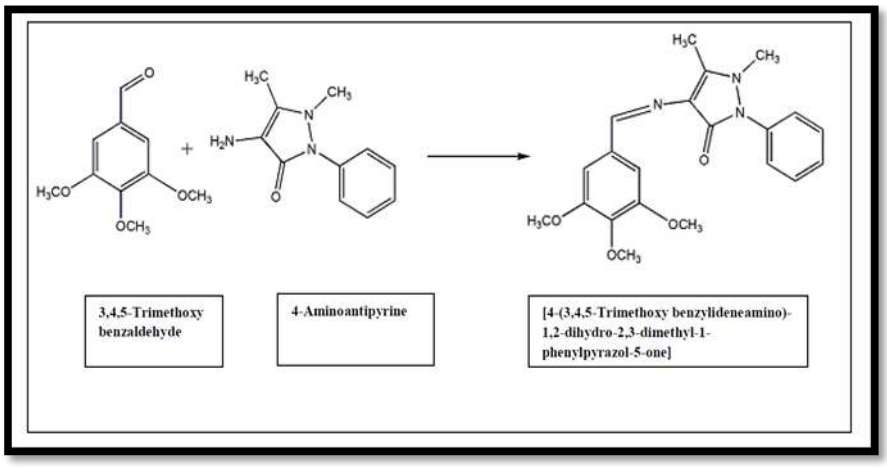


Figure 15: Graphical representation of antimicrobial activity of Mixed ligand Cd(II) Complex in terms of zone of inhibition against bacteria and fungi.



Scheme 1:Synthesis of Schiff base 4-(3,4,5-Trimethoxy benzylideneamino)-1,2- dihydro-2,3-dimethyl-1-phenylpyrazol-5-one[TMBAAP]

$$M.Cl_2.2H_2O + TMBAAP \rightarrow [M(TMBAAP)(H_2O)_2] + Try \rightarrow [M(TMBAAP)(Try)(H_2O)_2]$$

Scheme 2: Synthesis of Mixed ligand metal complex $[M(TMBAAP)(Try)(H_2O)_2]$ (M=Mn, and Cd)

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RESEARCH ARTICLE

Air Pollution and Its Influence in Human Health: A Public Perception Survey

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ABSTRACT

Air pollution is the largest environmental health risk that approximately kills 1 in 8 people globally, due to heart disease, stroke, respiratory disease and cancer. According to WHO air quality model (2016), about 92 % of world's population lives in places where air quality level exceeds the limits. When we breathe in dirty air or air pollutants deep into our lungs, it can cause new cases of a respiratory illness like Chronic Obstructive Pulmonary Disease (COPD), asthma and respiratory allergy. The aim of the study is to assess the impacts of air pollution in health and also the awareness level among the general population of Tiruchirappalli City Corporation. Totally 500 participants were included in the ten locations of the study area using simple random sampling method which was based on commercial zone, traffic zone and residential zone. The sample was taken from the road siders, vendors, drivers and households between the month of December 2019 and March 2021. The data were collected and it was analysed using SPSS 16.0 Version software. 61.6% participants have known about the importance of clean air, and 50.2% respondents have read frequently about air pollution. 75.6% respondents believed that the impact of air pollution was "high" and 66.1% responded that the impact of health due to air pollution is also " high". Based on the survey, the people have knowledge about air pollution and its health. During the interview, the public perception, traffic and more vehicles were main reasons for pollution and it creates health effects like chronic cough, asthma, eye irritation and skin problems. Based on the results of

the study, the awareness is raised among the public about the air pollution and the health impact. The Ariyamangalam Zone residents may be vulnerable to the respiratory diseases.

Keywords: Air pollution, Health, Perception, Diseases, Awareness

INTRODUCTION

Rapid growth of population, industrialization, urbanization, vehicular emission and various other human activities like residential cooking, lighting and heating, construction activities etc. emit huge amount of air pollutants to the atmosphere everyday (Amoatey *et.al.* 2020). As a result, air quality has degraded beyond its permissible limit in a large part of the world posing a serious threat to the environment. Particulate matter smaller than 2.5 μm in diameter is considered to be the best indicator of air quality and its health impacts (WHO, 2006, Lou, et. al, 2022). Hence, air pollution is an important stimulus for the development and exacerbation of respiratory diseases, such as asthma, chronic obstructive pulmonary disease, and lung cancer. There is generally less public awareness of its substantial impact on cardiovascular disease. Each 10 μg/m³ rise in PM2.5 concentration has been found to be associated with a 4%, 6% and 8% increased risk of all cause, cardiopulmonary and lung cancer mortality respectively (Feixang *et al.,* 2015). Estimate of annual premature mortality burden from chronic ambient PM2.5 exposure globally is quite large and alarming (Odonkor and Mahami, 2020, Liu *et al.,* 2017). The smaller a particle is, the more deeply it will penetrate to deposit on the respiratory tract at an increasing rate. In nasal-breathing, the cilia and the mucus act as a very effective filter for most particulates exceeding 10 μm in diameter. Because the coarse PM fraction settles quickly, it tends to lodge in the trachea (upper throat) or in the bronchi (Atkinson *et al.,* 2010). If we inhale this PM, it will be initially collected in our nose and throat. Then, our body will react to eliminate these intruding PM through such processes as sneezing and coughing (Kim, 2015, Gordon *et al,* 2018). It has been observed in many cities are in unhealthy status. Respiratory diseases are significant contributors to morbidity and premature mortality in India. The Global Burden of Disease of $PM_{2.5}$ exposure in India is one of the leading risk factors causing the premature death of approximately 1.67 million people. It has been estimated that 400,000 deaths have occured due to acute lower respiratory infection (ALRI) in children younger than five and 34,000 deaths because of chronic obstructive pulmonary disease (COPD) in women (Pantavou *et al.,* 2018).

Respiratory illnesses such as Chronic Obstructive Pulmonary Disease (COPD) and Asthma, act as a triggers of various forms of chronic interstitial lung diseases and lung cancer.(Arora *et. al,* 2018, Kurt *et al.,* 2016, Jiang *et al.,* 2016) . Both $PM_{2.5}$ and PM_{10} can cause serious respiratory damage with heart or lung disease, nonfatal heart attacks, irregular heartbeat, aggravated asthma, decreased lung function, and increased respiratory symptoms such as irritation of the airways, coughing, or difficulty breathing. (Srivastava, 2018). Lancet Glob Health 2018 studies states, accurate and comprehensive data on what is driving chronic respiratory disease burden in each state of India for improving respiratory health. COPD and asthma are the major risk in the different parts of India. The number of COPD cases in India has increased from 28.1 million in 1990 to 55.3 million in 2016.(Lancet Report, 2018).According to Lancet Report, 2019, 1·67 million (95% uncertainty interval 1·42–1·92) deaths were attributable to air pollution in India in 2019,accounting for 17·8% (15·8–19·5) of the total deaths in the country. The majority of these deaths were due to ambient particulate matter pollution (0·98 million [0·77–1·19]) and household air pollution (0·61 million [0·39–0·86]). The death rate due to household air pollution has decreased by 64·2% (52·2–74·2) from 1990 to 2019. This paper has revealed about the awareness of air pollution and also about the health issues among the public in the Tiruchirappalli city corporation through questionnaire based survey.

STUDY AREA

Tiruchirappalli city is the fourth largest corporation in the Tamil Nadu state, India. It is situated on the banks of the river Cauvery at 10°00′ to 11°30′N latitude and 77°45′ to 78°50′E longitude. Total geographical area of the city is 169.2 km^2 with the total population of 11, 82,000 in 2021. The average annual rainfall recorded is 747 mm.

METHODOLOGY

The study employed questionnaire to obtain quantitative data. The questionnaire was prepared both in local language and also in English for literacy reasons (Rajper et al., 2018). The study was conducted between the month of December 2019 and March 2021. The sample was carried out using simple random sampling method. The survey was conducted to measure the awareness about air pollution and also its health effects in ten location of the Tiruchirappalli city corporation. It was categorized as four zone such as Abishekapuram, Ariyamangalam, Ponmalai and Srirangam. Among the four-zones selected, Abishekapuram and Srirangam are the residential areas, Ariyamangalam is the commercial and industrial area, Ponmalai is the Traffic prone area. Mostly these zones are most congested with high population. 500 questionnaire were collected among the college students, road siders, householders, drivers, vendors and college students in the survey area. The sample was collected and it was analysed in the SPSS 16.0 software, the frequencies and percentage were obtained through the software as a result.

RESULTS AND DISCUSSION

Out of 500 participants, 273 (54.6%) were male and 227 (45.4%) were female. Majority of the participants were below 24 years age group with 215(43%), followed by 158 (31.6%) in 25-37 years age, 90 (18%) in 38-50 years age and 27 (5.4%) in 51-63 years age and 10 (2%) in more than 64 years age groups. Among the participants, 52 (10.4%) male have smoking habit and 77 (15.4%) male are addicted to alcohol. Among the respondents, 200 (40%) people have residence at K. Abishekapuram, 137 (27.4%) of them at Ariyamangalam, 87(17.4%) people at Ponmalai and 52 (10.4%) at Srirangam and 24 (4.8%) have in other areas. 308 (61.6%) respondents perceived the importance of air quality and 192 (38.4%) people did not perceive about the air pollution. When asked about the disease that are caused due to the exposure of air pollution, 303 (32.6%) people said asthma, 290 (31.2%) answered eye irritation, 171 (18.4%) said chronic cough, 88 (9.5%) answered skin problem and 78 (8.4%) replied heart diseases.

When asked about their exposure time in the traffic per day, 93 (18.6%) said that they were in traffic for 30 minutes and 104 (20.8%) for 1-2 hours, 141 (25.2%) for 2-4 hours, 162 (32.4%) had travelled for more than 4 hours. About the causes of air pollution, 426 (85.4%) people said the traffic jam, 280 (56.1%) and 269 (53.9%) respondents answered industries and rapid growth respectively. Among the participants 251 (50.2%) were read and heard about air pollution and 249 (49.8%) were not read and heard about air pollution. About the seasonal impact of air pollution 378 (75.6%) people said 'Yes' and 122 (24.4%) said "No". About the health impact of air pollution, 331 (66.1%) said "High" and 169 (33.9 %) said "Low". When questioned about the most polluted Zone in Tiruchirappalli city, the people replied that Ariyamangalam Zone (347 (45.1%) is the most polluted area followed by 128 (16.6%) K. Abishekapuram Zone, 166 (21.6%) Ponmalai Zone and 129 (16.8%) Srirangam Zone. This was shown in Table.1.

There were more male respondents than female. Young people were the major respondents in the survey. Most of them are residents of K. Abishekapuram Zone, a residential area. The awareness level is also more among the youngsters than the elders. Based on the survey, 61.6% respondents are aware of the importance of clean air and also the health impacts. It is observed the health effects are due to the air pollution. The average exposure of air pollution in the traffic area is above 4 hours. Around 33% of the respondents replied that the asthma and eye irritation were caused during the exposure of air pollution and also our study showed the causes of air pollution were emission of vehicular traffic jam and industries. The Ariyamangalam Zone includes the areas of Gandhi market, Palakkarai, BHEL, Thiruverumbur, Marakkadai, Palpannai and Kattur. These areas are more congested with heavy vehicle with more population and also Ariyamangalam having dumping sites are the reasons for the high pollution in this zone. Hence people in these areas are vulnerable to respiratory diseases. The Ponmalai zone is the second vulnerable zone which also includes more congested areas with higher traffic. Table.2 gives the data of the age wise awareness among the public about the importance of clean air, impact in the seasonal changes due to the air pollution, health impacts and reading about air pollution.

CONCLUSION

The study concluded that the most of the people were aware about the air pollution causes and its consequences over the period of time in human health. According to the public perception, Ariyamangalam zone is the highly vulnerable zone in Tiruchirappalli city. Around 33% of the respondents replied that asthma and eye irritation were caused mainly during the exposure of air pollution. The causes of air pollution are vehicle exhaust and industries Most of the respondents suggested that the government should take action and some of them expressed about tree plantations along the road sides to curb the air pollution.

RECOMMENDATIONS

- To reduce the burden of air pollution, we have to use public transports such as bus, train, carpooling, walking for short distances instead of using private vehicle, and keep automobiles well-tuned and maintained by using cleaner fuels (CNG).
- Government should educate the people about the risk of air pollution in the city by explaining the importance of preventive measures such as the use of face/respiratory masks (N-95 masks) and the use of eyeglasses/goggles to prevent the negative impacts of air pollution, to combat the situation.
- Creating awareness about air pollution through televisions, cell phones, and the Internet is an important action to get the knowledge associated health effects.
- Encouraging tree plantation in the society and helping in understanding the concept of reduce, reuse and recycle and emphasizing on use of clean energy sources should be done.
- Environmental protection agencies, and NGOs should adopt effective communication styles to educate the public and increase their awareness and understanding of the health risks associated with air pollution at individual, family, and community levels.

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Table.1: Basic Profile of Respondents

Profile	No. of Responses (n= 500)	In Percent (%)
Gender		
Male	273	54.6%
Female	227	45.4%
Age		
>24	215	43.0%
25-37	158	31.6%
38-50	90	18.0%
51-63	27	5.4%
< 64	10	2.0%
Smoking Habit		
Yes	52	10.4%
No	448	89.6%
Alcoholic		
Yes	77	15.4%
No	423	84.6%
Residence (in Zone)		
Abishekapuram	200	40.0%
Ariyamangalam	137	27.4%

Ponmalai	87	17.4%
Srirangam	52	10.4%
Others	24	4.8%
Is Clean Air Important?		
Yes	308	61.6%
No	192	38.4%
Diseases may cause due to exposure of air pollution?		
(Multiple Choice)	171	18.4%
Chronic Cough	303	32.6%
Asthma	78	8.4%
Heart Diseases	290	31.2%
Eye Irritation	88	9.5%
Skin Problem		
Average Exposure Time in Traffic per day?		
(Multiple Choice)	93	18.6%
30 Min	104	20.8%
1-2 hrs	141	25.2%
2-4 hrs	162	32.4%
Above 4 hrs		
Causes of air pollution (Multiple Choice)		
Traffic Jam	426	85.4%
Industries	280	56.1%
Rapid Growth	269	53.9%
Do you read and hear about air pollution, frequently?	251	50.2%
Yes	249	49.8%
No		
Impact of air pollution on seasonal changes	378	75.6%
Yes	122	24.4%
No		
Health Impacts		
High	331	66.1%
Low	169	33.9%
Most polluted places in Tiruchirappalli city (in Zone)		
(Multiple Choice)	347	45.1%
Ariyamangalam	128	16.6%
Abishekapuram	166	21.6%
Ponmalai	129	16.8%
Srirangam		

Table.2: Respondents Awareness of the Air Pollution and its Effects on Health

Age wise awareness among the Public	**Importance of Clean air**		**Air pollution impact on Seasonal Changes**		**Health Impact**		**Reading frequently about air pollution**	
Age	Known	Not Known	High	Low	High	Low	Yes	No
>24	127	88	158	57	133	82	94	121
25-37	99	59	122	36	107	50	88	70
38-50	59	31	70	20	64	27	48	42
51-63	16	11	20	7	21	6	14	13
< 64	7	3	8	2	6	4	5	5
Total (n=500)	308	192	378	122	331	169	249	251

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RESEARCH ARTICLE

Preliminary Phytochemical Analysis, Assessment of Total Flavonoid Content and Antioxidant Activity of Leaf of *Dianthus barbatus* L.

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ABSTRACT

Dianthus barbatus L., is that belongs to the Caryophyllaceae family. This plant has long been used in China, Japan, and Korea to cure wounds. Sweet Williams is one of the chefs' favorite edible flowers for salads, cakes, desserts, drinks, and trademark foods. In this study, we have screened the phytochemicals present in the plant for example, alkaloids, flavonoids, terpenoids, carbohydrates, glycosides, and tannins. The total flavonoid content was studied as a part of quantitative analysis. The total flavonoid content was determined using the Aluminium chloride method, which was estimated as 30mg quercetin equivalent/g of sample. In addition to this, the antioxidant property of the plant was also investigated by following the 2,2- Diphenyl1-1-picrylhydrazyl radical scavenging assay, and the IC_{50}value observed for the ascorbic acid of methanol solvent was 312.108 and for the sample was 530.166and also IC_{50} value observed for the ascorbic acid of acetone solvent was 591.230 and for the sample was 615.697.

Keywords: *Dianthus barbatus*, antioxidant, flavonoids, phytochemicals

INTRODUCTION

Dianthus barbatus L., sometimes known as Sweet William, is a winter annual that belongs to the Caryophyllaceae family and is frequently planted as a bedding plant for landscaping and cut flower production. a herbaceous short-lived perennial with upright angular stems that grows 30–60 cm tall. Leaves are green to glaucous, lanceolate, and 4–10 cm by 1–2 cm broad, with a sharp apex and a tapering base. Flowers (2–3.5 cm across) in clusters at the top of the stem; bracts 4 nearly as long as calyx tube, with membranous, ciliate margins; calyx tubular with acute teeth; petals 5 pink to red or purplish with a white base or variegated (Plates 1 and 2), limb ovate, bearded, and apex dentate; stamens slightly exserted, ovary Suboblong to an ovoid capsule, 4 valved, 1 cm wide, containing smooth, compressed brown ovoid seeds. In India, the area dedicated to flower seed production is around 800 hectares, with Himachal contributing approximately 2.60 ha valued at 4.11 lakh rupees. The climatic conditions in North India are ideal for the development of winter annual seeds. Farmers in many sections of the nation have accepted flower seed production as a powerful diversification option, as evidenced by the fact that farmers have reported 2.5–3 times higher economic gains from flower seed production than from wheat crops. This plant has long been used to treat wounds, gastrointestinal illnesses, and a number of other ailments in China, Japan, and Korea. Recent pharmacological studies have looked at the anticancer, antiviral, antibacterial, antifungal, and anti-insecticide activities of plants. One of the chefs' favorite food flowers is Sweet Williams. They are often used as a garnish for a range of salads, cakes, desserts, beverages, other signature dishes due to their ease of growing.

Although the Dianthus genus has over 320 species, only a handful have been studied for therapeutic potential. Dianthus spp. are mostly utilised in Chinese traditional medicine. In China, Dianthus caryophyllus, D. Chinensis, D. anatolicus, and D. barbatus are used, whilst D. basuticus is used in Africa, and D. superbus is used in China and Japan for a variety of diseases. Today, over 6,000 kinds of perennials are grown in the open field and utilised to landscape courtyards and in the private sector. Floriculture in Russian Federation territory is based on native types and foreign hybrids. *Dianthus barbatus* L. is a biennial ornamental plant known for its wide colour range and huge spherical inflorescences. The perfume of the flower is subtle, pleasant, and spicy. As the popularity of gardening has grown, there is a greater demand for hybrids with more beautiful flower shapes and a variety of colours. The environmental conditions and geographical location of the place are the determining variables in the establishment of any flower crop. Because of the wide range of plant development and flowering dates caused by natural environmental conditions, planting time cannot be predicted on a national basis. As a result, the planting period of a certain crop for a specific zone must be determined in order to get the greatest growth, blooming, and seed yields. As a result, experiments were carried out to standardise the planting period of sweet williamin order to achieve optimum flowering and seed output in the agro-climatic conditions of Himachal Pradesh's mid hills.

MATERIALS AND METHODS

Plant selection

The plant material was collected from the garden area of the Ahmedabad district of Gujrat state. The leaves were separated and washed with distilled water. Further, they were cut into small pieces and dried at room temperature. After drying, the plant material was made into a coarse powder using a grinder and stored in an air-tight plastic bag at room temperature.

Plant extracts preparation

The extracts were prepared by using cold extraction method (maceration technique). The dried powder of plant was dissolved in the solvent (methanol and acetone) using the ratio of 1:1 gm/ml in the conical flasks and kept in the orbital shaker for 24 hours. All the sample were separately filtered into petri-plates using the Whatman filter paper. The solvent was evoperated from the petri-plates and further stored at low temperature.

The extraction yield was evaluated by the standard formula-

Yield (%) = (Weight of dry extract ÷Weight of plant powder) ×100

Chemical and reagents

The chemical we used were bought locally from India i.e. we used 0.1% Ferric chloride, 1% aqeous hydrochloride, 20% sodium hydrochloride, chloroform, glacial acetic acid, acetic acid, concentrate H_2So_4 , 10% ammonium solution, 2% $FeCl_3$, 0,1% $FeCl_3$, 5% $FeCl_3$, 2% $CuSo_4$, ethanol, KOH pellets, 0.5% lead acetate, 10% lead acetate, 20% NaOH, Distill H_2So_4, distill water, Copper acetate, pyrine, 20% sodium nitroprusside, acetic anhydride solution, 10% $AlCl_3$, Quercetin, Gallic acid, 20% sodium carbonate, DPPH(1,1 diphenyl -2- picrylhydrazyl), Ascorbic acid. Other chemical reagent like Mayer's reagent, Wagner's reagent, Hager's reagent, Drangendorff's reagent, Molisch's reagent, Fehling's A and B, Barford's reagent, Benidict reagent, Millon's reagent, Folinciocalteu.

Qualitative phytochemical screening

To create a 1 mg/ml concentration of stock solution, 30 mg extract was dissolved in 30 ml methanol and acetone solvents separately. The presence of numerous secondary metabolites such as alkaloids, flavonoids, carbohydrates, proteins, lipids, glycosides, terpenoids, and saponins was determined by phytochemical analysis on both the methanolic and aectonic samples. The following approaches were used to conduct preliminary phytochemical screening.

Alkaloids

Mayer's test: 1mL extract was added to 2 mL Mayer's reagent; creamy precipitates suggest the presence of alkaloids.
Wagner's test: 1 mL extract was added to 1 mL Wagner's reagent, red brown color indicates that the alkaloids are present.
Hager's test: 1 mL extract was added to 1mL Hager's reagent, presence of yellow precipitates indicate presence of alkaloids.
Dragendorff's test: 1ml extract was added to 2mL Dragendorff's reagent; orange coloration shows presence of alkaloids.

Carbohydrates

Molisch's test: 1 mL extract was added to 1mL Molisch's reagent, formation of a violet ring signifies presence of carbohydrates.
Fehling's test: 1mL extract is treated with 1 mL Fehling's A and 1 mL Fehling's B reagent. It is boiled for 2 minutes. Red precipitates show presence of carbohydrates.
Barfoed's test: 1 mL extract is added to 1 mL Barfoed's reagent, it is boiled for 2 minutes; red precipitates are an indication for presence of carbohydrates.
Benedict's test: 1 mL extract is added to 1 mL Benedict's reagent, it is boiled for 2 minutes; colored precipitates show presence of carbohydrates.

Glycosides

Acetic acid test: 1mL filtrate is added to 2 mL chloroform, with 2 mL acetic acid, and drop-wise conc. H_2SO_4 is added to the test-tube, while simultaneously cooling on ice; violet to blue to green color change depicts the presence of glycosides.
Ferric chloride test: 1 mL filtrate is treated with 1 mL glacial acetic acid, and 1 mL 2% $FeCl_3$ solution, this is added to a test-tube containing 1 mL of conc. H_2SO_4, it forms 2 layers, the upper layer having red brown color, and the lower layer having blue green color signifies the presence of glycosides.

Proteins

Millon' test: 1 mL extract is treated with 1 mL Millon's reagent; white color shows presence of proteins.

Copper sulphate test: 1 mL extract is added to 1 mL 2% Cu_2SO_4, 1 mL ethanol, and 1 pellet of KOH, the ethanol layer has pink color which will signify presence of proteins.

Phenols

Ferric chloride test: 1 mL extract is added to 1 mL 5% $FeCl_3$; dark green color shows presence of phenols.

Lead acetate test: 1mL extract is added to 0.5 mL lead acetate; white orecipitation shows presence of phenols.

Folin Ciocalteu test: 1 mL extract is added to 1 mL FolinCiocalteu reagent; blue green color indicates presence of phenols.

Flavonoids

Sodium hydroxide test: 1 mL extract is added to 3 mL 2% NaOH, turns yellow, 1 mL dil. H_2SO_4 is added; yellow color will disappear which signifies the presence of flavonoids.

Lead acetate test: 1 mL extract is treated with a few drops of 10% lead acetate solution; yellow precipitates show flavonoid presence.

H_2SO_4 test: 1 ml extract is added few drops of H_2SO_4, orange color precipitation shows flavonoid presence.

Saponins

Foaming test: 1 mL extract is added to 20 mL distilled water, and it was shaken vigorously; if foam appears, it shows presence of saponins.

Fixed oils and fats

Oil stain check: extract is poured drop-wise on a filter paper, if oil stains are observed; it signifies presence of fixed oils.

Terpenoids

Copper acetate test: 1 mL extract is treated with 1-2 drops of copper acetate solution; emerald green precipitation defines presence of terpenoids.

Chloroform test: 1 mL extract is treated with 2 mL chloroform and 3 mL of conc. H_2SO_4, it forms a layer, if there is a formation of red brown color ring it shows presence of terpenoids.

Cardiac glycosides

Sodium nitroprusside test: 2 mL extract is added to 1 mL pyridine and 1 mL 20% sodium nitroprusside; pink or red color depicts presence of cardiac glycosides.

Steroids:

Salkowaski's test: 2 mL extract is shaked with 1 mL chloroform, and conc. H_2SO_4 is added side by side, red color shows presence of steroids.

Liebermann Burchard's test: 1 mL extract is treated with 2 mL acetic anhydride solution and 2 mL H_2SO_4; violet or green color signifies presence ofsteroids.

Tanin test

Lead acetate test: 1 ml extract is added in 1 ml 0.1% lead acetate; yellow precipitate shows presence of tannin.

$FeCl_3$ test: 1 ml extract is added to 0.1% $Fecl_3$, brown-green or black color shows presence of tannin.

Quantitative analysis

In the methanol solvent, the stock solution was produced at a concentration of 1 mg/ml. To prepare the stock solution, 30 mg of crude extract was dissolved in 30 ml (methanol and acetone) in the case of the sample. By adding the stock solution to the solvent, a concentration gradient series of 100g/ml to 1000g/ml was created. the standard solution (quercetin for TFC and Ascorbic acid for DPPH assay and acetate buffer for FRAP assay) were prepared similarly with the same concentration of 1 mg/ml standard solution of methanol and acetone solution.

Total flavonoid content

The total flavonoid content of a plant sample is determined using the aluminium chloride colorimetric technique. A normal series is conducted here as well, although the standard flavonoid that we utilise is Quercetin, rather than total phenolic concentration. Quercetin solution is made in methanol at a concentration of 1mg/mL. The stock solution series is created by combining quercetin solution with methanol at concentrations ranging from 100g/mL to 1000g/mL. In the test tubes, 0.1mL of 10% aluminium chloride is added, followed by 0.1mL of1M potassium acetate solution and 23.8mL of distilled water. It is well shaken and incubated for 30 minutes before the absorbance is measured using a spectrophotometer at =415nm. The regular series is performed in threes. The process is carried out in triplicates, utilising sample extract to provide an average value that may be replaced from the equation obtained by plotting the calibration curve of the standard series. The total flavonoid content of a sample is expressed in milligrams Quercetin equivalent/gram (mg QE/g) using the following formula:

QE= C x V/M Where,
C = concentration of gallic acid obtained from the calibration curve in mg/ml
V = volume of the extract solution in ml
M = Weight of the extract in g

Antioxidant activity

DPPH free radical scavenging assay

Antioxidant activity can be determined by a lot of methods. We have used the DPPH radical scavenging assay. DPPH is 2, 2 diphenyl-1-picrylhydrazyl. 0.01mM DPPH is prepared 0.01mM in methanol, i.e., 0.004% DPPH solution. The standard compound used for antioxidant activity is ascorbic acid as 1mg/1mL concentration. Stock solution is prepared ranging from 200µg/mL to 1000µg/mL concentration, and it is replaced with 1mg/1mL sample extract solution when the sample is under process. To the test tubes containing stock solution, 1mL of 0.004% DPPH solution is added and it is shaked well before incubating in dark for 20-30 minutes. Each test tube is prepared in triplicates to minimize errors. DPPH is a photosensitive chemical that means it will degrade in presence of light, therefore the whole procedure is done in a dark room with utmost precision and the test tubes are covered in aluminium foils for more security. Once incubated, the absorbance is measured using a spectrophotometer at λ=517nm. The scavenging activity is calculated based on the percentage of DPPH radical scavenged (%) with the help of the following formula,

$$\% \text{ Inhibition Activity} = \frac{\text{Absorbance of the blank - Absorbance of sample}}{\text{Absorbance of blank}} \times 100$$

The values of %I are used to calculate IC50 values which represent the half maximal inhibitory concentration that is a measure of effectiveness of the substance in inhibiting the specific biological or biochemical function.

FRAP Assay

Determination of Ferric Reducing Antioxidant power (FRAP) is based on the ability of the sample to reduce Fe to Fe ions. At low pH, in the presence of TPTZ, ferric-tripyridyltriazine (Fe – TPTZ) complex is reduced to the ferrous (Fe – TPTZ) from with the formation of intense blue colour having an absorption maximum at 593nm. A solution of 0.0248gm TPTZ in 0.024 ml HCl and 0.0258gm ferric chloride powder was diluted in mili q water, all dissolved in 80ml sodium acetate buffer (pH 3.6) at a ratio of 1:1:10. Aliquots of extract solutions 1 ml were added to 4 mL of the FRAP solution and were allowed to react for 30 min at 37°C before reading the absorbance at 593 nm. This method follows 4mL of FRAP reagent was mixed with 1 mL of the aqueous extract at different concentration (1mg/ml). the mixture was then incubated at 37°C for 30 min in the dark. The absorbance was measured at 593nm against a blank having all the reagents excluding the sample using spectrophotometer. 1 ml of methanolic extract with concentration range from 100 g/ml to 500 g/ml was taken in test tubes. Quercetin in the same concentration range (100 g/ml to 1000 g/ml) was used as a positive control.

Statistical analysis

To avoid any mistakes when completing all of the tests, each value obtained is the sum of the three replicates. The mean standard error is used to represent all of the results. The IC50 value was calculated, as well as all other statistical analyses, using the most recent version of graphpad prism.

RESULTS

Yield value: the yieldvalue is used in the quantification of the phytoconstituents with respect to the crude extract. The methanolic extract had a yield of 2.68% while the yield observed for acetone extract was 3.40%. the formula used was;

Yield (%) = (weight of dry extract ÷ weight of plant powder) ×100

Calculation:

%Yield for methanolic extract = (0.4023÷15) × 100

%Yield for acetone extract = (0.340 ÷ 10) × 100

Result for phytochemical screening

Alkaloids, carbohydrates, glycosides, flavonoids, saponins, fixed oil and lipids, tannins, and terpenoids were found in a methanolic extract of *Dianthus barbatus* leaf samples. The acetone extract, on the other hand, revealed the same incidence as the methanol extract. This finding shows that polar and non-polar solvents extract the same secondary metabolites. Acetone is a solvent that may dissolve both non-polar and polar compounds. As a result, the activity in both solvents is the same (table 1). The table represents the results of the phytochemical tests performed. '+' sign indicates the presence and '-'sign indicates the absence of the respective phytochemicals.

Result for total flavonoid content

The AlCl3 colorimetric technique is used to determine the total flavonoid content because AlCl3 forms an acid stable compound with the C4 keto group or the C3/ C5 hydroxyl group of flavonoids or flavones. Running the standard, Quercetin, yielded the calibration curve.

y= 0.0015x+ 0.0445

R2=0.9716, The total flavonoid content of the plant sample was estimated using the aforementioned equation and found to be mg QE/g of sample.

Result for antioxidant activity

DPPH radical scavenging test was used to determine the plant's antioxidant capacity. For assessing the antioxidant activity of plant samples, DPPH is the most widely utilised reagent. For colorimetric analysis, it is a quick and efficient procedure. The antioxidant drug lowers DPPH, which is shown by a shift in colour from purple to yellow and a decrease in the absorbance at 517nm, as measured by a spectrophotometer. Ascorbic acid was employed as the reference antioxidant agent for comparison in the investigation of D. barbatus leaves antioxidant activity. The results were presented in terms of the 50 percent inhibition concentration (IC_{50}), which was determined using a regression equation generated by drawing a graph of concentration versus percent inhibition. The value of $IC_{50}$is inversely proportional to the antioxidant activity, means the lower value of $IC_{50,}$ the stronger is the antioxidant activity. In this study, the value of $IC_{50}$for standard, ascorbic acid was evaluated to be 312.108 and that of the leaf methanol was found to be 530.166and also the value of IC_{50} for acetone standard, ascorbic acid was evaluated to be 591.230 and that of the leaf acetone sample was found to be 615.697.

RESULT OF FRAP ASSAY

In terms of preparing the chemicals for the working solution, the FRAP assay is less time consuming and difficult. It is a simple and low-cost procedure that does not necessitate the use of any special chemicals. For all concentrations, the results obtained in FRAP are shown to be repeatable. As a result, FRAP is a good approach for determining antioxidant activity Thaipong *et al.* found that the FRAP test demonstrated good repeatability, was straightforward, and could be conducted quickly in research done in 2006 for assessing antioxidant activity from guava fruit extracts. in this method the result for leaf methanol is 1.072±0.014 and also result for leaf acetone is 0.627±0.015. this result shown that the antioxidant property is present in this sample.

DISCUSSION

Traditional knowledge and basic medications derived from plants are becoming increasingly important as modern interventions expand. The phytochemical screening and diverse bioactivities indicate that the plants might be used to treat a variety of illnesses and disorders. There isn't enough research on *Dianthus barbatus* to demonstrate its value. The current study aims to investigate the secondary metabolites of plants, as well as their quantitative evaluation and antioxidant properties. The antioxidant property protects the cell from free radical damage, which can protect the human body from many life-threatening illnesses such as cancer. The study of plants obtained from diverse agro-climatic zones can aid in evaluating the plant's overall qualities and chemical makeup, as well as making the best use of it to battle a variety of illnesses. Saponins, carbohydrates, glycosides, flavonoids, sterols, anthraquinones, polyphenols, tannins, fatty acids, and anti-oxidant, anti-inflammatory, wound-healing qualities have been found in the aerial sections of *Dianthus barbatus*. We determined that the IC50 value of *Dianthus barbatus* is 530.166 of leaf methanol solvent and 615.697 of leaf acetone solvent based on our findings.

CONCLUSION

The study's main goal was to look for numerous secondary metabolites in *Dianthus barbatus* leaves, quantify them, and determine their antioxidant properties. Because there isn't enough research based on the plant with in-depth understanding of the metabolites, chemicals, and activities contained in the plant, *Dianthus barbatus* was chosen to study its potentials. The presence of numerous major Phyto-constituents indicates that the plant has some important pharmacological properties that might lead to future medical uses. The antioxidant capacity of the plant is demonstrated by the DPPH radical scavenging experiment. There is a link between the plant's phenol content and its antioxidant action. Carbohydrates, flavonoids, terpenoids, and cardiac glycosides are among the phytochemicals found in the plant. Further research into phytochemicals may aid in detecting the plant *Dianthus barbatus*'s anticancer, antibacterial, anti-inflammatory, antifungal, and anti-diabetic properties. Older research demonstrate traditional therapeutic benefits that can be utilised now by investigating all of the plant *Dianthus barbatus*' characteristics. The contents and activities of West Zone vary, making it necessary to research the wide range of activities that the plant obtains in order to improve the plant's applications and utilizations in other industries.

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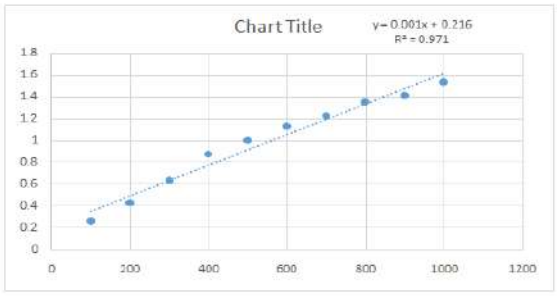
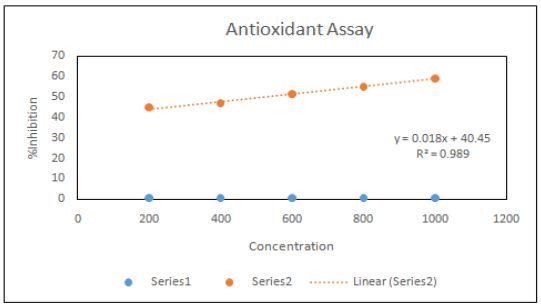
Table1: represents the results of the phytochemical tests performed. '+' sign indicates the presence and '-'sign indicates the absence of the respective phytochemicals

Sr.no	Phytochemical	Test	General observation	Result	
				Methanol	Acetone
1.	Alkaloids	Mayer's test – 1 ml filtrate +mayer's reagent.	White creamy ppt.	_	_
		Wagner's test- 1 ml filtrate + wagner's reagent.	Red brown ppt.	_	_
		Dragendroff's test - 1 ml filtrate + Dragendroff's reagent.	Orange ppt.	+	+
		Hager's test- 1 ml filtrate + Hanger's reagent.	Yellow ppt.	_	_
2.	carbohydrates	Molish test-1ml filtrate + molish reagent	Violet ring	+	+
		Fehaling's test-1ml filtrate + fehaling A reagent +fehaling B	Red ppt.	+	-
		reagent, boil for 2min			
		Barfoed's test- 1mL filtrate + Barfoed's reagent, boil for 2 min	Red ppt	+	_
		Benedict's test- 1mL filtrate + benedict reagent, boil for 2 min	Colored ppt.	_	_
3.	Glycosides	1mL filtrate + 2mL chloroform + 2mL acetic acid + conc. H_2SO_4 (cooling it on ice)	violet Blue Green	_	_
		1mL filtrate + 1mL H_2SO_4	Redish orange coloration	+	+
		1mL filtrate + glacial acetic acid + 2% $FeCl_3$, add this into test-tube containing conc. H_2SO_4	Upper layerred brown Lower layerblue green	+	_
4.	Proteins	Millon's test- 1mL filtrate + millon's reagent	White coloration	_	+
		1mL filtrate + 2% $CuSO_4$ + ethanol + KOH pellet.	Pink color of the ethanol layer	_	_
5.	Phenolics	1mL filtrate + 5% $FeCl_3$	Dark green color	_	_
		1mL filtrate + 0.5mL lead acetat	White ppt	-	_
		1mL filtrate + FolinCoicalteau reagent	Blue green color	_	_
6.	Flavonoids	Extract + 3mL 2% NaOH (turns yellow) + dil. H_2SO_4	Yellow color disappears	+	+
		Extract + few drops 10% lead acetate	Yellow ppt.	+	+
7.	Saponins	Extract + 20mL D.W. & shake	Presence of foam	+	+
8.	Fats and fixed oils	A drop of extract on filter paper	Oil stains of filter paper	+	+

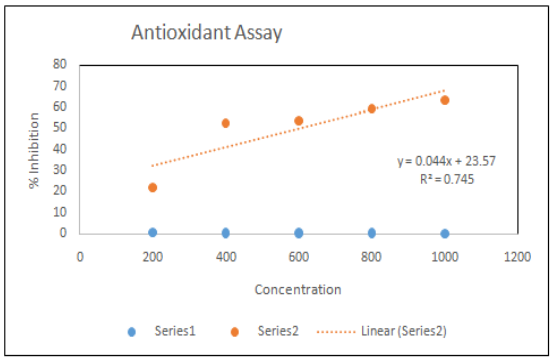
9.	Terpenoids	Extract + 1-2drops copper acetate solution	Emerald green ppt.	+	+
		Extract + 2mL chloroform + 3mL conc. H_2SO_4, forms a layer.	Formation of red brown colored ring	-	+
10.	Cardiac glycosides	2mL filtrate + 1mL pyridine + 1mL 20% sodium nitroprusside	Pink or red coloration	+	_
11.	Steroids	Salkowaski's test: 2mL extract + shake with chloroform + add conc. H_2SO_4 side by side	Red coloration	_	_
		Liebermann Burchard's test: 1mL filtrate + 2mL acetic anhydride solution + 2mL H_2SO_4	Violet or green coloration	_	_



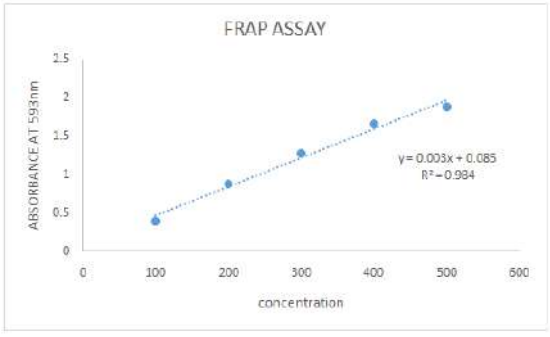
Graph 1: graph showing the standard curve of quercetin for total flavonoid content



Graph 2: showing result of Antioxidant Assay of Leaf Methanol



Graph 3: the result of Antioxidant Assay of Leaf Acetone



Graph4 : the result of antioxidant of FRAP Assay

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RESEARCH ARTICLE

In vitro Investigations on the Anticancer Activity of Various Solvent Extracts *Allamanda cathartica* Human Liver Cancer Cell Line (HepG2)

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ABSTRACT

Allamanda cathartica Linn. (Apocynaceae) is a perennial shrub that grows in a variety of habitats across the world. The phytochemical and cytotoxic properties of *Allamanda cathartica* leaf extracts were investigated in this study. At room temperature, hexane, chloroform, and methanol were used to extract the leaves of the plant *Allamanda cathartica.* Phytochemical screening was used to identify the different types of chemicals in the extracts. The extracts were also put through an MTT experiment to see if they were cytotoxic to HepG2 cells. The hexane extract has the least amount of cytotoxic action. Chloroform extract had an IC50 value of 336.09 ± 1.30 and methanol extract had a modest activity of 462.91± 8.35 percent. Studies on cytomorphology have also been conducted out. The study's findings suggest that *Allamanda cathartica* leaf extracts have great therapeutic potential.

Keywords: *Allamandacathartica*, leaf extracts, phytochemical studies, MTT assay, HepG2 cell line, Cytomorphology studies.

INTRODUCTION

According to the world health organization (WHO) report (WHO Cancer fact sheet, 2017), around 13% of the total deaths are found to occur due to an appalling syndrome called cancer. It is anticipated that the death toll from this heinous illness would exceed 13.1 million by 2030. [1] Chemoprevention strategies have been used to slow the carcinogenesis process by using either natural or synthetic substances. (Sporn et al., 1976). [2] Although enormous research works are devoted to advancing the cancer treatment procedure, still serious limitations prevail. Firstly, the cost for the chemoprevention method is exorbitant for all economic status of people to afford. Secondly, the side-effects associated with the aforementioned treatment are enduring for a long period of time. Thus, there is a dire need for developing novel and effective chemotherapeutic drugs that are both cost-effective and possess decreased

side-effects. In recent times, the extracts from plants were found to satisfy both the aforementioned characteristic features while still being efficient toward cancer treatment (Pezzuto, 1997; Wu et al., 2002).[3,4] However, this approach of using plant extracts as therapeutic drugs for treating various diseases, including cancer, has been in practice since antiquity called as traditional medicines (Cragg and Newman, 2009).[5] At present, traditional medicines constitute more than half of the contemporary chemotherapeutics used for treatment of cancer (Newman and Cragg, 2012).[6] Particularly, India with a vast diversity in the vegetation (housing 9 preeminent botanical zones), has been a pioneer in utilizing traditional medicines with valuable and enriching chemical constituents (Chatterjee, 1962). [7] Around 80% of the world population is found to be dependent on the use of traditional medicines for treating various diseases according to the estimates of WHO (Modern Ghana.com, 2017).[8] In this regard, the flowers, leaves and roots of *Allamandac athartica* belonging to the family of Apocynaceae has been utilized in some countries in the tropical regions as traditional medicine for treating jaundice, abdominal pain, insect bites, malaria, controlling vomiting, splenomegaly and also as laxatives. It has also been demonstrated to possess antitumor and anticancer activity against HIV and pathogenic fungi.[9] There are also studies substantiating the wound healing properties of the plant. [10]Since 1954, the chemical contents of A. cathartica have been intensively researched. [11] Alkaloids were discovered in preliminary chemical investigations. [12] flavonoids, Steroids [13], tannins [14, 13] phenolic compouns [13] saponins [14, 13] anthraquinones [15], carbohydrates [16], anthocynins [17], coumarin [18], carotenoids [19], carbohydrates [16], glycosides [14], hydrocarbon [16], quinones [18], and terpenes [18, 13] extracted from a variety of sources, primarily leaves,shoots, flowers, roots, stems and stem bark. Thus, the key objective of the current work is to screen the phytochemical constituents of the leaf extracts of *Allamanda cathartica* and to explore their invitro cytotoxic activities.

MATERIALS AND PROCEDURES

Plant-based materials

Fresh leaves of *Allamanda catharitica* were acquired in the Loyola College campus in Chennai, India, and confirmed by one of the DDERI 1-5 voucher specimens, which were prepared and put in the Entomology Research Institute of Loyola College Chennai.

Plant extraction

The leaves of the plant were dried in the shade and then crushed. 1 kg of ground material was mixed with hexane, chloroform, and methanol in a glass percolator and maintained at room temperature for 24 hours. The percolates were collected and filtered after the extraction method was performed five times. The extract was weighed after being concentrated under decreased pressure in a rotating evaporator at 50°C. To be included in the results.

Preliminary Phytochemical analysis

The following phytochemical analysis were carried out according to standard procedures [20, 21].

Test for Alkaloids: 2ml of 1 percent HCl was added to 1ml of leaf extract before boiling for a few minutes. After boiling, 2-3 drops of Drangendroff's reagent was added and the sample was observed for reddish brown precipitate.

Flavonoid test: 1 ml of 10% NaOH was added to 2.5 ml of leaf extract. Drops of conc. HCl were introduced from the test tube's side. The fading of yellow color to white, indicatesthe presence of flavonoids.

Test for Terpenoids

At the test tube walls, 400µl of chloroform and 4-5 drops of conc.H2SO4 were added to 1 ml of extract. Terpenoids are indicated by the presence of a reddish brown ring.

Test for Tannin

1mL of extract was heated with a few drops of $FeCl_3$. The color of the sample was examined for blue, black, or green.

]

Test for Saponin

1ml of leaf extract was mixed with 2ml of $NaHCO_3$, and the solution was thoroughly agitated to produce the froth that shows the presence of Saponin.

Test for Anthraquinone

2ml of 5% KOH was added to 1ml of leaf extract, and the pink coloration indicates the presence of Anthraquinone.

Test for Cardiac glycoside

Some few drops of FeCl3 and conc.H_2SO_4 were introduced from the side panels of the test tube to 2.ml of extract premixed using 2ml of glacial acetic acid. The existence of cardiac glycoside is indicated by the appearance of a reddish brown ring.

Test for Starch:

500 µl of iodine was added to 1ml of extract, resulting in a blue color indicating the presence of starch.

Cytotoxicity assay

As previously stated, cell viability was measured using a standard colorimetric Sulforhodamine B (SBR) test. (22)

Cell culture

Human liver cancer cell line (HepG2) was obtained from the National Centre for Cell Sciences (NCCS, Pune India). The cells were cultured as a monolayer in Dulbecco's Modified Eagles Medium (DMEM, Himedia) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (1 mg/mL) in complete tissue culture medium. The cells were grown and maintained in a CO_2 incubator at 37°C in a mixture of 5% CO_2 and 95% air with 90% relative humidity. The cells were trypsinized and passaged when they reached 90-98 percent confluence.

Cell viability assay

The cytotoxicity of was determined using the MTT colorimetric test. hexane, chloroform, and methanol extracts of *Allamanda catharitica*. In a 96 well microtiter plate, concentrations of 100 µl,200 µl,300 µl,400 µl, and 500 µl/ml of the three extracts were applied to $1x10^5$ cells/ml medium 200 µl medium and cultured for 48 hours. After the media was withdrawn, each well was filled with MTT 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide solution, 100µl/ml (5mg/ml in PBS) and incubated for 4 hours. Appearance of a purple formazan derivative was observed using inverted microscopy. After agitation for 5 minutes, the MTT solution was decanted and the formazan crystals were dissolved in 100 µl DMSO. The absorbance was measured at 570 nm in an ELISA microplate reader. The cell viability percent was calculated as followed = OD of control- OD of tested sample / OD of control x 100.

Cytomorphological Studies

The cell morphological alterations of HePG2 cells treated with hexane, chloroform, and methanol extracts were evaluated. HepG2 cells were plated on 100 mm plates and incubated for 24 hours. Fresh hexane, chloroform, and methanol extracts were applied at varying concentrations (100µg/ml 200µg/ml, 300µg/ml, 400µg/ml, and 500µg/ml) and incubated for 24 hours after the culture medium was withdrawn. At 40°C, the incubated HepG2 cells were seen under an inverted microscope.

Statistical analysis

Statistical analysis was performed on the findings of the MTT colorimetric experiment. The experiments were conducted in triplicate, and the findings have been reported as men ± standard division. Graph pad prism version 5 software was used to do a one-way analysis of variance (Anonava).

RESULTS

MTT colorimetric test of HepG2 cells was used to determine the cytotoxic activity of three consecutive extracts of the Leaf portion of the plant, namely hexane, chloroform, and methanol. The findings are shown in table 2 and Fig 4 - 6.The IC $_{50}$ values of the three extracts were determined to be as follows. Hexane ≥ 500μg/ml, chloroform 336.09 μg/ml and methanol 462.91μg/ml. respectively.

The HepG2 cell line's morphological profile

Figures 7a-7f, 8a-8f, and 9a-9f show the morphological alterations of HepG2 cells treated with various doses of hexane chloroform and methanol extracts. According to the morphological characteristics of the different extracts, the hexane extract is the most efficient in preventing HepG2 cell development. As the concentration of the extract was raised, the cells became lysed and destroyed. The chloroform extract had the most cytotoxic potential among the extracts, as shown by the cell viability testing.

DISCUSSION

In every country on the planet, cancer is the main cause of mortality and a major impediment to extending life expectancy.[23] According to World Health Organization (WHO) projections for 2019, [24]. Many traditional plants have demonstrated their worth as a source of varied chemicals with varying therapeutic potentials, and they continue to be an important field for the development of innovative medications for the treatment of a variety of ailments. [25] The screening and isolation of crude extracts from plants has been the mainstay of traditional approaches for discovering natural products. [26]. Herbal medicinal compounds may be found in abundance in the *Allamanda cathartica.* These extracts and chemicals from the *Allamanda catharitica* contain a wide spectrum of antifungal, antiviral, and antibacterial properties, according to pharmacological research. [27] anticancer [28] diabetes [29] diuretic and emetic [30] fever [31] jaundice [32] malaria [33] parasitosis [34] rheumatism [35] splenomegaly [34] and snake bites [36], Leaves, stem bark, flowers, roots stem, sap, seeds, and branches are the most often utilized plant components, in order of frequency. The cytotoxic efficacy of methanol and aqueous extracts of leaves at doses of 10, 5, 2.5, 1.25, and 0.6 mg/mL on BHK-21 cells was not observed. [37]. P388 leukemia cells had an IC50 of 85 _g/mL after being treated with methanol extracts from leaves in another investigation. [38]. its usage of silver nanoparticles ($AgNO_3$) in conjunction with *A.cathartica* aqueous latex samples demonstrated a pharmacological impact on human mononuclear blood cells.[39]. Their LD50 values for methanol, ethyl acetate, petroleum ether, and chloroform extracts of *A. cathartica* leaves were 111.61, 131.14, 332.42, and 47.86 _g/mL, correspondingly, against *Artemia salina*. [40]. Compounds (142), (139), and (138) isolated from 95 percent ethanol leaf extracts inhibited human nasopharnyx carcinoma (KB) cells in vitro, with LD50 values of 2.1,2.6, and 2.7 μg/mL, respectively [41]. As a result, the presence of this molecule may be to accountable for the cytotoxicity found in our research.

CONCLUSION

According to the findings of this investigation, basedon MTT results, *Allamanda cathartica* has significant cytotoxic activity on several extracts of HepG2 cell lines, indicating that it is a good source of active molecules against cancer.

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Table 1 Preliminary phytochemical screening of the *Allamanda catharitica*extracts

S. No	Constituent of the phytochemical	The Results of the Test		
		Hexane	Chloroform	Methanol
1	Alkaloid	-	-	-
2	Flavonoid	-	+	+
3	Terpenoids	+	+	-
4	Tannin	-	-	+

5	Saponin	-	+	+
6	Anthraquinone	-	-	-
7	Cardiac glycosides	+	-	+
8	Starch	-	-	-

Table 2.The cytotoxicity of different extracts from *Allamanda catharitica* Leaf component against the HepG2 cell line.

Concentration µg/ml	Viability of cells as a percentage		
	Hexane	Chloroform	Methanol
100	95.45 ± 0.79	88.58 ± 0.91	93.48 ± 0.66
200	89.53 ± .088	75.33 ± 0.95	83.07 ± 2.76
300	83.71 ± 0.90	64.55 ± 0.82	73.00 ± 2.73
400	76.14 ± 1.32	49.23 ± 0.36	60.61 ± 2.15
500	69.54 ± 1.02	37.68 ± 0.84	48.41 ± 1.26

Figure 1: The aerial Plant parts of *Allamantha cathartica*

Figure 2: Leaves of *A. cathartica*

Figure 3: Flower of *A. cathartica*

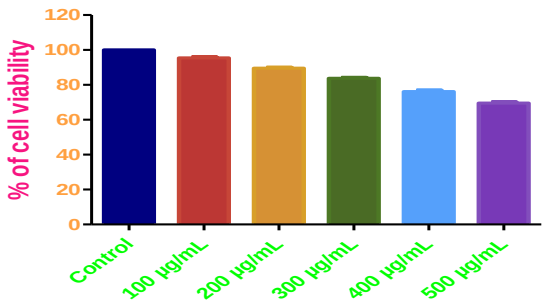


Figure 4: Cytotoxic activity of *Allamanda catharitica* hexane extract against HepG2 cells.

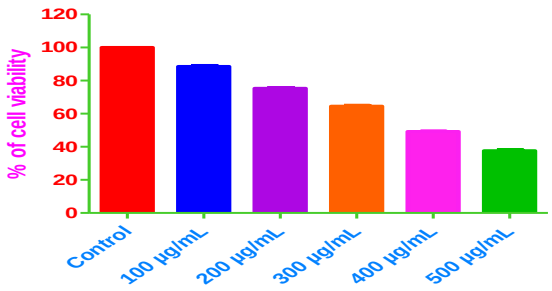


Figure 5: Cytotoxic activity of *Allamanda catharitica* chloroform extract against HepG2 cells.

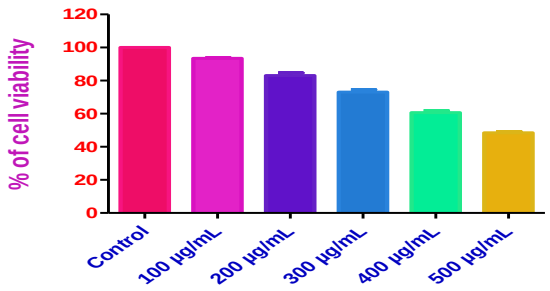
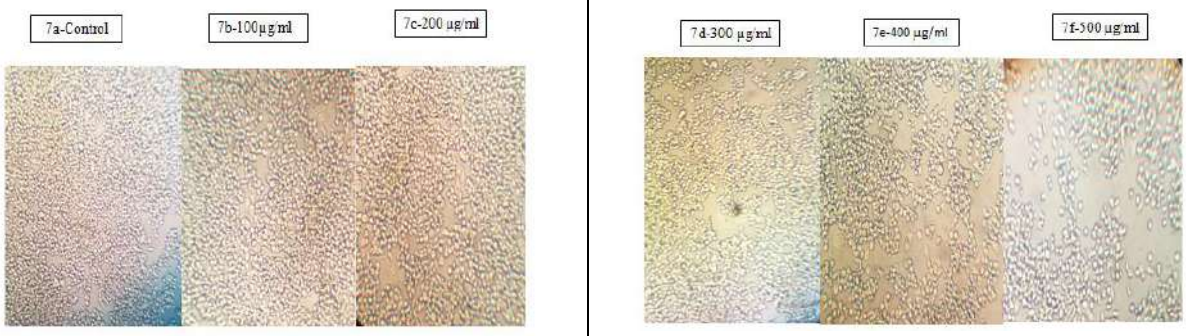
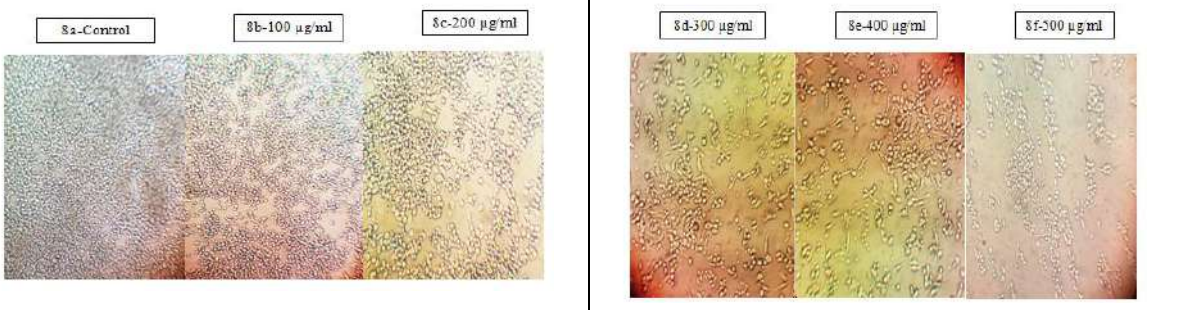


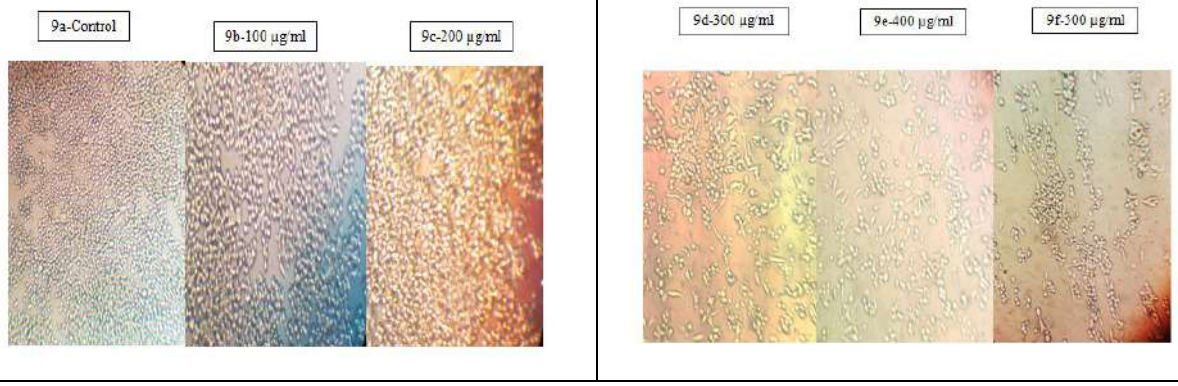
Figure 6: Cytotoxic activity of *Allamanda catharitica*m ethanol extract against HepG2 cells.



Figures 7:A through 7f Control, 100 g/ml, 200 g/ml, 300 g/ml, 400 g/ml, and 500 g/ml morphological profile of HepG2 cells following treatment with hexane extract of *A. cathartica*



Figures 8:A through 8f Control, 100 g/ml, 200 g/ml, 300 g/ml, 400 g/ml, and 500 g/ml morphological profile of HepG2 cells following treatment with chloroform extract of *A. cathartica*



Figures 9:A through 9f. Control, 100 g/ml, 200 g/ml, 300 g/ml, 400 g/ml, and 500 g/ml morphological profile of HepG2 cells following treatment with Methanol extract of *A. cathartica*

RESEARCH ARTICLE

Efficacy of Organic and Inorganic Product on Seed Yield and Seed Quality in Blackgram through Seed Treatment

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ABSTRACT

The present experiment was carried out to study the effect of seed hardening treatment on seed yield and seed quality in blackgram (*Vigna mungo* (L.) Hepper). The seeds of blackgram VBN 6 were hardened with chemicals like 1% $ZnSO_4$, 1% $MnSO_4$, 1% $CaCl_2$, 1% KCl, 1% KNO_3 and organics like 5% cow dung + 5% Cow urine, 10% Cow dung, 10% Cow urine and 5% Cow dung + 5% Goat dung along with control. All the treated and untreated seeds were evaluated for seed yield and yield contributing characters under field condition and seed quality of harvested seeds under lab condition. From the field evaluation, it was observed that 1% $ZnSO_4$ hardening treated seeds recorded higher values for the growth and yield attributing characters. The resultant seed of 1% $ZnSO_4$ hardened plant registered significantly higher values of quality parameters when compared to other treatments and control. From the present study, it was found that seeds hardened with 1% $ZnSO_4$ performed better than all other treatments in seed yield and seed quality.

Keywords: Zinc sulphate, Cow dung, Seed hardening, Seed yield, Blackgram.

INTRODUCTION

Blackgram (*Vigna mungo* (L.) Hepper), is mainly cultivated in Indian subcontinent. In India blackgram is popular as "Urad dal" and it is highly prized pulse among all the pulses. In India, the area, production and productivity of pulse were 24.91 million hectares, 16.35 million tones and 733 kg per hectare, respectively. In general, pulses give lower yield than cereals. This led to the assumption that pulses may have a lower genetic potential for yield than cereals. Lower productivity is due to the reason that pulses are grown mostly under marginal and rainfall areas. The main constraint in raising the productivity levels of pulses in dry lands are the inadequate of soil moisture, poor

fertility status of the soil, lack of quality seed of high yielding varieties/hybrids, poor keeping quality and lack of storage facilities [1].In dry land agriculture, drought resistance of plant is one of the very important factors to get the higher yield. Though this is largely depends on genetic makeup of the variety, pre-sowing treatments like 'hardening' is also practiced to defy the ill effect of drought on emergence and growth of crop. Seed hardening is conditioning of seed to withstand adverse environment and adaptive conditions. It is a creation of resistance in the seed for better outgrowth of seedling. Such physiological reorganization is induced by hydration and dehydration processes [2]. With the above facts, the current study was carried out to assess the effect of pre-sowing seed treatment on seed yield and seed quality in blackgram.

MATERIALS AND METHODS

Genetically and physically pure seeds of blackgram VBN 6 were given hardening treatment with the following chemicals and organics viz., T0 – Control, T1 – 5% Cow dung + 5% Cow urine, T2 – 1% ZnsO4, T3 – 1% MnSO4, T4 – 1% CaCl2, T5 – 1% KCl, T6 – 1% KNO3, T7 – 10% Cow dung, T8 – 10% Cow urine, T9 – 5% Cow dung + 5% Goat dung. During pre-sowing seed treatment, seeds were soaked in the respective chemical solutions and organics for 12 hours at the ratio of 1:1 of the seeds. After soaking, seeds were dried back to the original moisture content. The field experiment was conducted with the above treatment by adopting RBD with three replications. Observation on growth and yield parameters were recorded for each treatment replication wise. The recommended package of practices was adopted for raising the crop. The data collected were subjected to statistical analysis as described by Panse and Sukhatme [3].

RESULTS AND DISCUSSION

In field study, when compared to other treatments, (T_2) 1% $ZnSO_4$ recorded significantly higher values for all the characters studied namely plant height (28.11 cm), number of branches per plant (5.00), number of nodules per plant (10.00), number of clusters per plant (11.67), number of pods per plant (25.33), pod length (6.14 cm), pod yield per plant (7.29), number of seed per pod (6.00), hundred seed weight (4.44 g) and seed yield per plant (5.23 g) (Table 1). The improvement in vegetative growth parameters plant height, number of branches per plant, number of nodules per plant might be due the cumulative effect of hardening and $ZnSO_4$ could have triggered the biosynthesis of nucleic acids, proteins and consequential enhancement of cell division besides the enhanced metabolic activity of the plant resulting in the increased uptake of nutrients which are associated with improved crop growth [4]. It might also be due to the role of zinc involvement increased cell division strengthening of cell wall and cell enlargement which have a plant growth promoting capabilities and often applied as exogenous plant growth enhancer [5]. Among the treatment T_2 – 1% $ZnSO_4$ recorded the more number of nodules per plant which might be due to improved mobilization of nutrient. $ZnSO_4$ also plays a major role during the early stage of Rhizobium-Legume symbiosis nodule factors produced by rhizobia, in response to legume produced flavonoids. The similar results were reported by Sathiyanarayanan *et al.* [6] and Reka [7].

In field study among the treatments, (T_2) 1% $ZnSO_4$ recorded significantly higher values for yield characters namely, number of clusters per plant, number of pods per plant, pod length, pod yield per plant, number of seed per pod, hundred seed weight and seed yield per plant over the control. The probable reasons for improvement in yield attributes might be due to the hardening chemicals which accelerate the synthesis of protein, nucleic acid, bound water content, repair mechanism and growth of seedling resulted in increasing uptake of nutrients and ability of treated plants to unfavourable condition when compared to control. The improved weight (100 seed weight) from the T_2 hardened seed might be results of improved photo assimilation and its translocation and partitioning from source towards the sinks [8,9]. In contrast, the control registered the minimum values for yield attributing characters including number of pods per plant, pod yield per plant, number of seeds per pod and 100 seed weight might be due to the slow starch hydrolysis due to the poor availability of water and curtailed emergence of seedling seems to be relative to inefficient mobilization and utilization of seed resources [10]. From the present study it was that evident the

control plants have a poor plant establishment, poor vegetative growth, which results in lesser photosynthesis and reduced translocation of photo assimilates from sources to sink. In any seed production program, seeds from harvest until next sowing season are prime important to ensure good seed germination and plant stand. Seed is hygroscopic in nature. After harvest, fresh seeds are dried back to safer original moisture content (10 per cent) and evaluated the seed quality parameters of resultant seeds under the laboratory condition. In laboratory condition, resultant seed (T_2) 1% $ZnSO_4$ recorded significantly higher values for all the quality parameters studied namely germination percentage (92%), speed of germination (14.39), root length (18.51 cm), shoot length (24.93 cm), seedling length (43.45 cm), dry matter production (0.32 g/10 seedlings), vigour index I (4011.95) and vigour index II (29.87) (Fig. 1). The improved seed quality of resultant seed might be due to the more food reserved materials in seed and reduced stress condition during seed maturation and development favoured this positive effect. In might be due to the enhanced crop stands, growth and yield that ultimately results in the improvement of seed quality [9,11]. From the present study, it was found that seeds hardened with 1% $ZnSO_4$ performed better than all other treatments in seed yield and seed quality of blackgram.

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Table 1 Effect of seed hardening treatment on growth and yield parameters in blackgram

Treatments	Plant height (cm)	Number of branches per plant	Number of nodules per plant	Number of clusters per plant	Number of pods per pant	Pod length (cm)	Pod yield per plant	Number of seed per pod	Hundred seed weight (g)	Seed yield per plant (g)
T_0	23.77	2.33	7.33	9.33	23.33	4.73	6.59	4.33	3.96	4.46
T_1	26.53	3.33	8.00	10.33	24.00	5.17	7.02	4.67	4.09	4.54
T_2	28.11	5.00	10.00	11.67	25.33	6.14	7.29	6.00	4.44	5.23
T_3	26.52	3.67	7.67	10.00	24.33	4.89	6.60	4.67	3.99	4.84
T_4	26.58	3.33	7.33	9.67	24.00	5.01	6.86	4.67	3.96	4.68
T_5	25.91	3.67	7.67	10.00	24.33	5.12	6.74	4.67	4.03	4.59
T_6	25.80	3.33	8.00	10.00	23.67	4.92	6.74	4.33	4.12	4.62
T_7	28.03	4.00	9.00	10.67	24.33	6.03	7.15	5.00	4.27	5.19
T_8	25.81	3.67	7.67	10.00	23.67	4.76	6.77	5.33	3.96	4.66
T_9	26.09	3.33	7.67	9.33	24.00	4.87	6.71	5.33	4.15	4.63
Mean	23.31	3.56	8.03	10.10	24.10	5.16	6.84	4.90	4.09	4.74
SEd	0.006	0.15	0.01	0.04	0.10	0.006	0.006	0.04	0.01	0.01
C.D (P = 0.05)	0.0084	0.31	0.025	0.091	0.21	0.0085	0.013	0.09	0.02	0.03

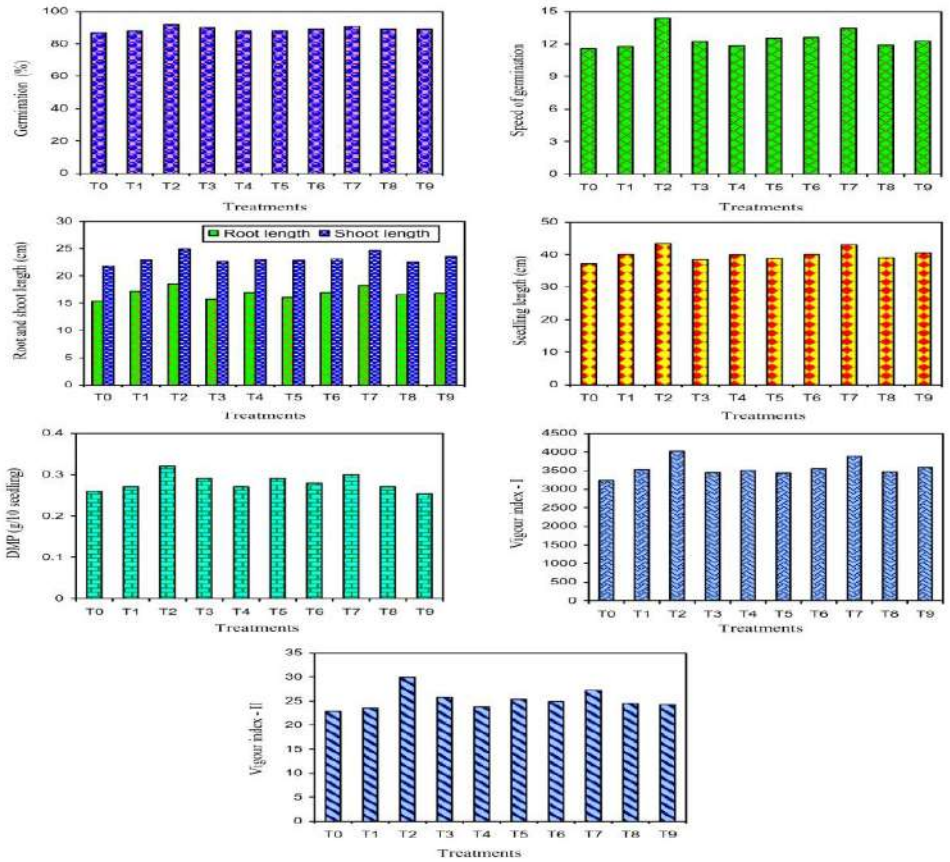

Fig. 1. Effect of seed hardening treatment on resultant seed quality parameters in blackgram

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RESEARCH ARTICLE

Brain Tumour Detection System

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ABSTRACT

One image will express the concept clearly than explaining it with more than 1000 words. Important thing is that the image should be clear and precise. A MRI scanned image can help the physician to diagnose the problems easily. Main aim of this work is to produce clear image after denoising using DTCWT based on local adaptive denoising and hybrid filtering is proposed. PSNR and MSE values were calculated and it is better than exisiting methods. It is then segmented using nano tree segmentation which is combined with improved multi class SVM classification for better results. Finally the system will identify whether the image contains a tumour or not. And also classifies the stage of brain tumour as Normal, Begin, Moderate and Severe stage.

Keywords: DTCWT, SVM, PSNR, MSE, Denoising, Segmentation, classification

INTRODUCTION

Image processing is used to improve the quality of the image for better human understanding. MRI images are best method for identifying the abnormal tissue growth in brain whereas CT scan is best for bone problems. Soft tissues related problems will be shown best in MRI scan. Quality of the image is not maintained because of noise. Sometimes there is a possibility that because of noise the physician may diagnose MRI image wrongly. Image noise is random variation of brightness or colour information in image which was not present in the object imaged[1].Noise reduction has to be done prior to all image processing techniques. An image may contain many types of noises such as shot noise, thermal noise, Gaussian noise, pepper noise, speckle noise etc. To address noise related problems in digital image processing, image filtering technique has generally been applied to restore the originality of the image. The aim of denoising is to preserve the actual information of the image and to remove unwanted information in the image. Mostly Gaussian noise are additive in nature. Shot noises are neither additive nor multiplicative in nature. So we can apply proper procedure to remove those noises. Physical noise comes from the corpuscular nature of light.

Physical noise includes dark shot noise and photon shot noise [2].Each noise can be removed by separate method. So initially image denoising should be done before processing the image data. In this work, combinations of median filters are used to remove multi-type noise. Noise may originate from noise sources present in the vicinity of image capturing devices, faulty memory location or may be introduced due to imperfection or inaccuracy in the image capturing devices such as misaligned lenses, weak focal length, scattering and other adverse conditions of environment [3]. Mean filter is used for noise reduction using mean of neighbourhood. Median filter is used for reducing noise using median of neighbourhood whereas Laplacian of Gaussian filter is a edge detection filter. This work proposes a hybrid median filter to remove multi-type noises and to produce low mean square error.

DUAL TREE COMPLEX WAVELET TRANSFORM (DTCWT)

This method is an extension of Discrete Wavelet Transform (DWT). A quantization scheme that improves the compression ratio and quality of the reconstructed image was proposed. After quantization, a model for Denoising using DTCWT based on local adaptive denoising and hybrid filtering is proposed. In this research, a hybrid median filter has been implemented in the original image to produce enhanced image. Multi-type noise can be reduced at this level. Dual tree complex wavelet transform are formed with enhanced image. In formulation the DTCWT is complex pair of real and imaginary discrete wavelet transform trees. Finally, enhanced image is applied with local adaptive image denoising method. Dual tree complex wavelet transform are formed with enhanced image. In formulation the DTCWT is complex pair of real and imaginary discrete wavelet transform trees [4]. The real and imaginary part is explained in fig 2 and fig 3. Finally, enhanced image is applied with local adaptive image denoising method. The human visual system (HVS) is more sensitive to changes in the achromatic plane (brightness), than chromatic ones [5]. The analysis and synthesis filters are well discussed by Kingsbury provide a perfect reconstruction analysis and synthesis filter banks for image denoising with critically-sampled for discrete wavelet shrinkage [6]. The results were compared with various filters and with existing method DWT. This method combines the approach of filtering and Wavelet transformations for Denoising at various levels. The results are compared with various types of noise and different level of noise variances. Several parameters are evaluated like PSNR, MSE are calculated and it seems PSNR values are comparatively high than the existing method. It also proves that, MSE values are very less which means lower the error rate and higher the clarity of image. So the PSNR value of the proposed algorithm is observed for different levels and seems to be higher than the existing model DWT. This method preserves the edge boundaries while denoising.

NANO TREE SEGMENTATION

After denoising, a Segmentation based on Nano-Tree segmentation was used. A signal passes through two filters, one is high pass filter and other is low pass filter. Then the signal will be decomposed into two parts, one is approximation image with low frequency and other is detailed image with high frequency. At every level of decomposition the image will be divided into four parts such as one approximation image and remaining are three detailed images such as horizontal, vertical and diagonal images. Decomposition of the image is done to any number of levels as the user wants. We will have the entire image as single region. Then divide the region if they dissimilar color, shape, texture etc. If the pixel values are closer to neighbour pixel values then it considered as single region and its predicate value is true or if pixel values are different then its predicate is assigned as false and divide the region into nine pieces called Nano tree. This is splitting and merging of an image. Nano tree segmentation belongs to region based segmentation. It uses the concept of region merge and splitting concept. The purpose of Nano tree Segmentation is to store data of points on a two dimensional space. This method will find set of pixels from the neighbour called seed points and finds the pixel can be divided into a group of seed point or not [8].

Algorithm

- In this tree, each node should have nine children.
- Construction of Nano tree

– Taking approximation image produced from TWT stage as input image for this stage.

– Divide the current two dimensional space into multiples of 3. (perform number of rows/cols mod 3, if remainder is one, then include two rows and cols in the matrix. if remainder is two, then include one row and column in the matrix.
– If the image has no color or value then the box does not contain any points. So no child for it.
– Create a child object for each box if it contains any points in the image and store it in two dimensional space.

Repeat the above two steps for each of the children

Segmentation will divide the image into several regions and preserve the edges. Better results can be obtained by removing noise in each portion of image. In this way, we can find whether to include the pixel or not in the region. At the same time, we can also determine that the corresponding each pixel belongs to boundary (edges) or textures. If it belongs to the boundary then the values will be preserved for processing. Consider the entire image as single region. Then divide the region if they dissimilar color, shape, texture etc. If the pixel values are closer to neighbour pixel values then it considered as single region and its predicate value is true or if pixel values are different then its predicate is assigned as false and divide the region into nine pieces called nano tree. This is splitting and merging of an image. Usually the image will be divided into four sub regions but in this paper we are dividing it into nine sub regions which will give more accuracy in segmentation. In the fig 6, the children 9 has decomposed. So the node 9 got expanded. Otherwise it won't expand. For example if the children 92 got pixel information then it will get expand which is explained in fig 7. The image is produced using nano tree segmentation (3X3). Dividing the matrix into (4X4) matrix then computation will get more intense. So nano (3X3) tree segmentation is the best for segmentation.

IMPROVED SUPPORT VECTOR MACHINE

After segmentation, an improved multi class Support Vector Machine for classification is proposed. This will classify the output as Normal, Begin, Moderate and severe stages of the Brain. Mostly MRI images are used by the physician to diagnose whether tumor is present or not. SVM uses so much of time in training the dataset. It can be used to solve both classification and regression type of problems. It will represent the data as points and divide them into groups with maximised margins. So it is very good in high dimensional spaces. There are four important types of SVM. Two of them are for regression types and two of them are for classification SVM. Classification consists of two important steps. Training and Testing. Grouping of homogenous category data and the numerical properties are analyzed [9]. Based on the properties of image, training class created. In continuous next testing phase, image features are classified using these feature-space partitions. To automatically segment data, clustering algorithms are used for unsupervised algorithm whereas statistical processes are used for supervised algorithms. The constructing training classes should have the following properties are independent, discriminatory and reliable. Support vectors are the points closest to the line for both circles and stars. Then we should find margin by computing the distance between line and support vectors. SVM algorithm will create hyperplane and separates data into classes. Support vector machine algorithm will actually classify the mixed data into different classes (class will have similar data). If three dimension data are used for analysis then two dimensions should be used for separating them into two parts. N-1 dimensions are used for separation in N dimensional space. There are three hyperplanes are available such as A, B, and C [10]. Among these three, choose the right hyperplane to classify stars and circles.

From Fig 9, find the right hyperplane in such a way that each class will divide well. From Fig 13, it is clear that hyperplane A is having the maximum boundary but SVM will choose the hyperplane which accurately prior to maximizing margin. So the optimal hyperplane in this scenario is B. From Fig 14, it is clear that one circle lies in the area of star class as an outlier. SVM will not bother about outliers instead it will find the hyperplane tat has maximum margin. Support vector machine is machine learning supervised algorithms that analyze data used for classification or regression [11]. Mapping of input data to particular group by making use of algorithm is the purpose of classification [12]. Fig 15 refers to the improved SVM working model for brain tumour detection system. Principal Component Analysis(PCA) is mostly used for the purpose of exploratory data analysis which analyzes data that has multiple variables. So dimensionality reduction takes place at PCA [13]. Principle Component Analysis (PCA) [14] is used to reduce the dimensionality of data i.e. reduced features. PCA can perform well if the training set is small when compared with feature extraction [15]. Middle level image processing usually deals with segmentation, edge

detection, shape recognition, etc [16], [17].The proposed methodology will acquire the image, Pre Processing, morphological operations like skull striping, segmentation and feature extraction by improved SVM. The images in the SVM classifier are processed under two steps: training and testing. Mostly the images are acquired from the laboratories and converted into digital images. The digital images are acquired by MATLAB software is the completion of first step acquiring images. The quality of the image can be enhanced by adjusting the brightness of the pixel value. Sometimes images can be resized to 256X256 in the process of image enhancement. Finally this work combines the Preprocessing, segmentation and classification techniques to identify the brain tumour stage. The final step of the process is classification meant for feature extraction. The improved SVM classifier will classify the images into four classes like, Normal, Begin, Moderate and Severe stage.

RESULTS AND DISCUSSIONS

Fig 16. Final Brain Detection system. The validation parameters like accuracy, specificity and sensitivity were calculated. Additional parameters like Mean, standard deviation, variance, skew, contrast, energy, auto correlation and homogeneity were also calculated. The proposed system is identifying the severity of Tumour with better accuracy than other system.

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Table 1. Different types of filters with their PSNR values

sno	Types of Filters	PSNR (in db)
1.	Motion blurred	16.72
2.	Wiener filter	17.89
3.	Median filter	14.12

Table 2. MSE & PSNR values for DWT & DTCWT

Sno	Noise	Noise variance (σ)	MSE value (DTCWT)	MSE value (DTCWT)	PSNR Value (DWT)	PSNR Value (DTCWT)
1.	Salt and pepper	10	62.34	60.11	43.77	45.84
2.	speckle	20	55.25	52.74	38.97	40.12
3.	Poisson noise	25	43.21	42.17	37.68	39.94
4.	Gaussian noise	30	47.81	43.23	29.78	32.14

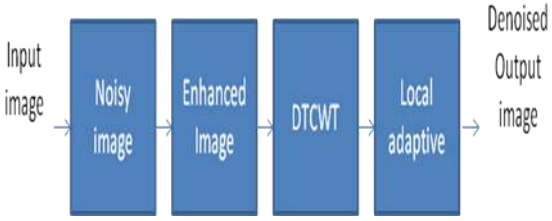

Fig 1. Image Denoising based on DTCWT using Local adaptive Denoising

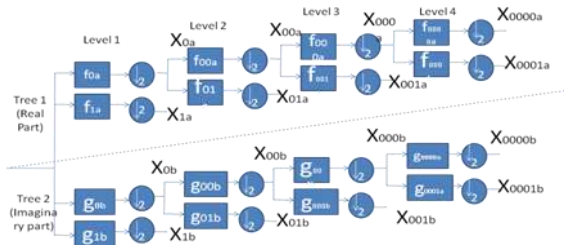

Fig 2. Analysis Phase of DTCWT

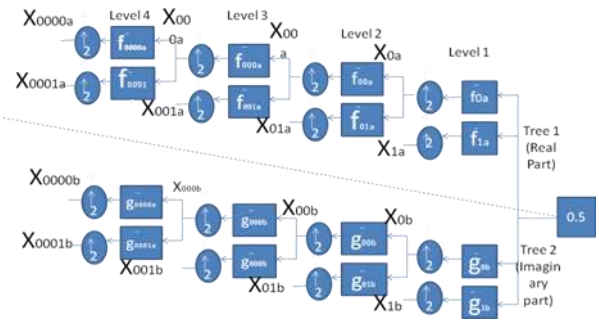

Fig 3. Synthesis Phase of DTCWT

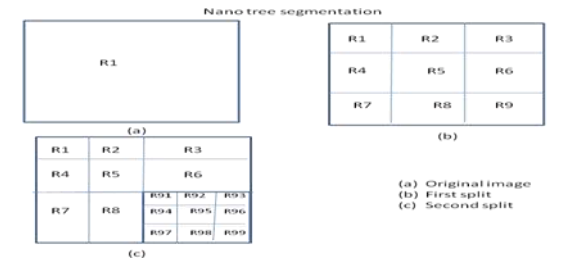

Fig 4. a) Original image b) first split c) second split

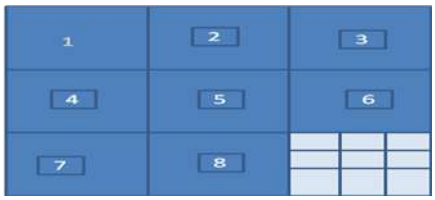

Fig5:Nano tree segmentation second split

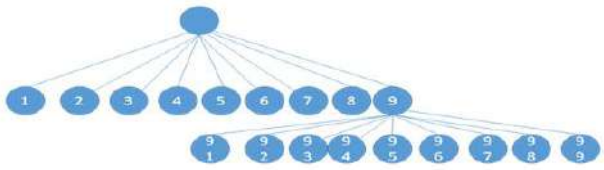

Fig 6: Data structure representation of Nano tree segmentation

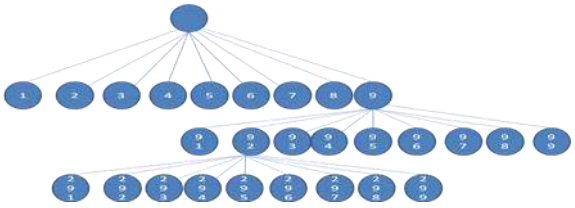

Fig 7. Nano tree segmentation, level 3

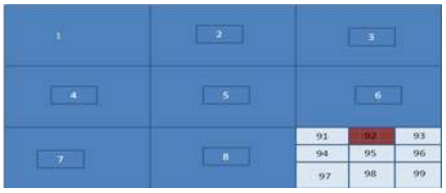

Fig 8. Nano tree segmentation at level 3

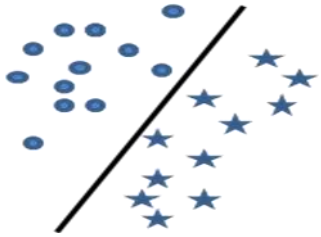

Fig 9. Group similar items

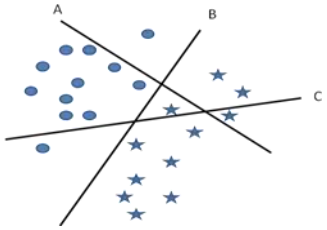

Fig 10. Identify right hyper plane(scenario-1)

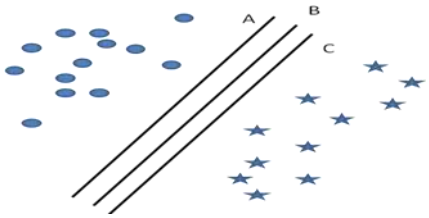

Fig 11. Identify right hyper plane (scenario-2)

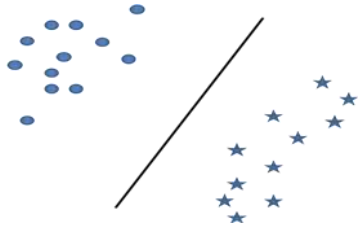

Fig 12. Maximised Boundary of margin

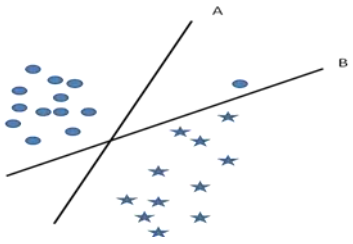

Fig 13. Identify right hyperplane(scenario-3)

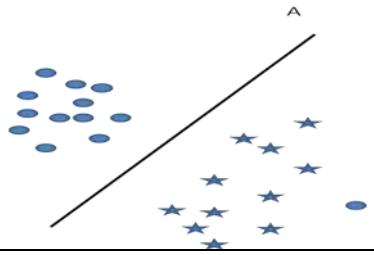

Fig 14. Identify right hyperplane(scenario-4)

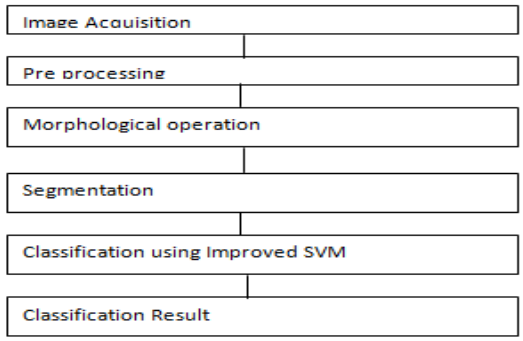

Fig 15. Improved SVM Working Model

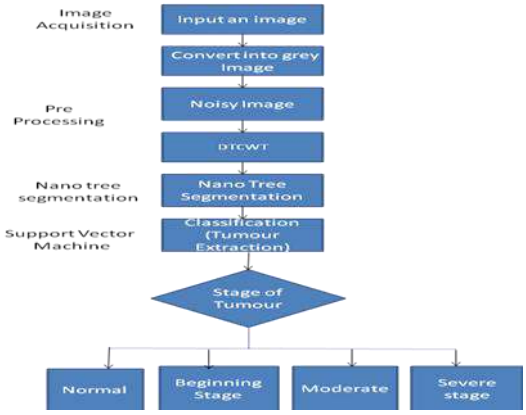

Fig 16. Final Brain Detection system

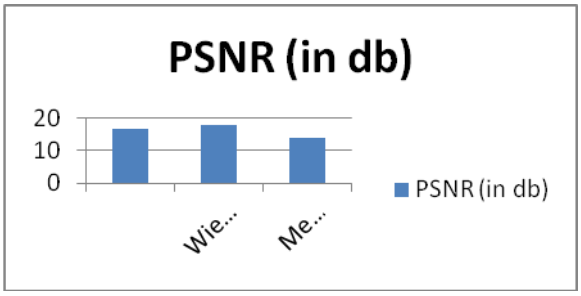

Fig 17. Comparison of various filters with their PSNR values

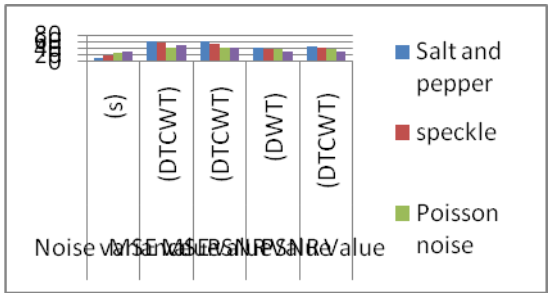

Fig 18. Comparison of MSE & PSNR values for DWT & DTCWT

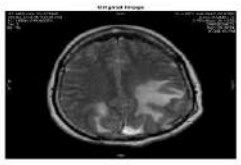

Fig 19. Original image

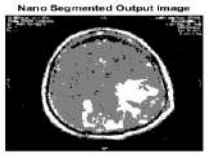

Fig 20. Segmented Image

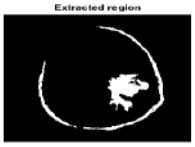

Fig 21. Extracted Image

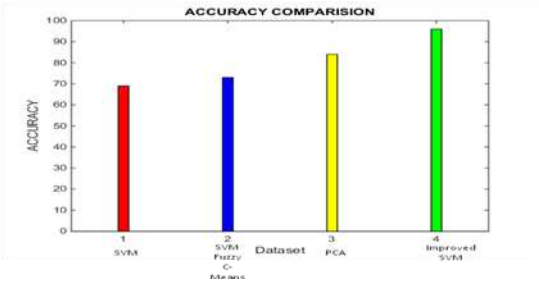

Fig 22. Graph for Accuracy comparison with Other models

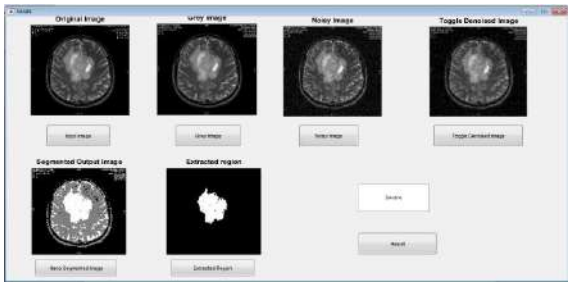

Fig 23. Sample output for severe brain tumour

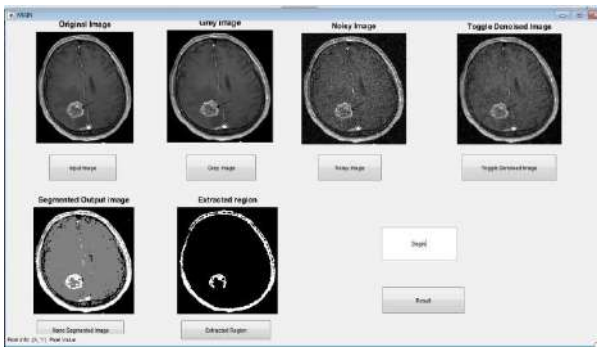

Fig 24. Sample output for Begining stage of brain tumour

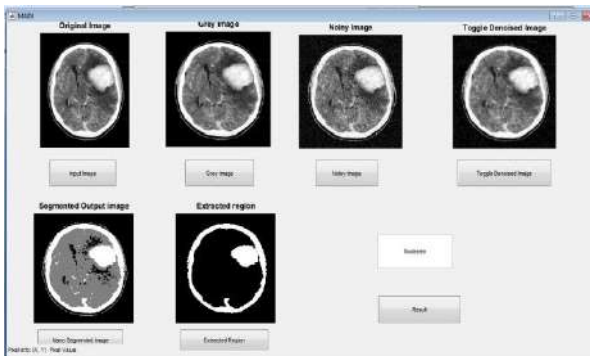

Fig 25. Sample output for Begining stage of Moderate tumour

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RESEARCH ARTICLE

Role of Foliar Spray on Crop Growth, Seed Yield and Seed Quality in Rice

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ABSTRACT

The experiment was carried to evaluate the effect of foliar application on crop growth, seed yield and seed quality in rice. The foliar applications were given with organic and inorganic product *viz.,* 3% Panchagavya, 15% seaweed extract, 2% DAP, 1% Boric acid, 0.5% $ZnSO_4$ along with control. From the experiment results, it was revealed that foliar application with 1% recorded higher values for the growth and yield parameters *viz.,* days to first flowering, days to 50 per cent flowering, plant height, panicle length, leaf length, leaf breadth, number of tillers per plant, number of productive tillers per plant, number of seeds per panicle, seed L/B ratio, seed yield per plant, dry matter production and 1000 seed weight. The resultant seeds from the same foliar applied plants registered the best seed quality parameters.

Keywords: Foliar application, Boron, Seed yield, Seed quality, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important staple food, as it was estimated that 40% of the world's population mostly use rice as a major source of energy. Rice occupies about 11% of world's agricultural land and ranks second in terms of cultivated area (Tumrani *et al*., 2015). India ranks first in area and second in production. In Tamil Nadu, area under rice cultivation is 17.21 lakh hectares with production of 61.32 LMT and productivity of 4.43 metric tons per hectare (2018-2019 Government of Tamil Nadu). A good quality seed can have a positive effect on yield and quality of the crop. Quality seed plays seminal role in augmenting agricultural productivity as well as production. Only by using quality seeds, productivity can be enhanced to the tune of 15-20%. Seed of high viability and vigor is essential for attaining high yield and quality.

Foliar application is a technique of feeding plants by applying liquid nutrients directly to their leaves. The fertilizers can be botanicals or chemicals. Plants can absorb essential elements through their leaves. The absorption takes place through their stomata and through their epidermis. Foliar spray of nutrients at critical growth stages will improve the emergence of panicle and reduce sterility. Foliar application of micronutrient is a simple way for making quick correction of plant nutrient status. It boosts the process responsible for potential yield of crops such as nitrogen metabolism, uptake of N and protein, photosynthesis, carbonic anhydrase activity, resistant to abiotic and biotic stresses and protection against oxidative damage (Kulhare *et al*., 2017).Foliar application of nutrients plays a vital role in rice production by stimulating the root development, energy transformation, various metabolic processes, translocation activity in plants, thereby increase the yield. Foliar fertilization is high effectiveness, rapid plant responses and elimination or reduction of toxicity symptoms brought about by excessive soil accumulation of the elements (Rajasekar *et al*., 2017). With this background, the present study was designed to evaluate the effect of foliar application on crop growth, seed yield and seed quality in rice.

MATERIALS AND METHOD

A field trial was conducted at experimental farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai university during year 2020 by adopting Randomized Bock Design (RBD) with three replications. The crop was raised with the spacing of 20 × 10 cm and recommended package of practices for rice were followed. The solution was sprayed on rice foliage at critical growth stages *viz.,* tillering stage, booting stage and milking stage by using knapsack sprayer.

Foliar treatments

T_0 – Control
T_1 – 3% Panchagavya
T_2 – 15% Seaweed extract
T_3 – 2% DAP
T_4 – 1% Boric acid
T_5 – 0.5% $ZnSO_4$

During the growth period, five randomly selected plants were tagged in each treatment replication wise, and the growth attributing characters, and yield attributing characters were recorded under the field condition. After harvest, the resultant seeds were pooled, cleaned, dried to a moisture content of 12% and graded using BSS sieve for uniformity. The randomly selected samples of seed from each treatment were evaluated for their seed quality characters. The data were analyzed statistically adopting the procedure described by Panse and Sukhatme, 1985.

RESULT AND DISCUSSION

From the present field trial, significant results were obtained in foliar nutrients applied plants. Among the treatments, 1% applied plants (T_4) recorded higher values for the growth *viz.* days to first flowering (71 DAS), days to 50 percent flowering (74 DAS), plant height (78.34 cm), panicle length (24.20 cm), leaf length (33.00 cm), leaf breadth (1.17 cm), number of tillers per plant (28), and yield parameters *viz.,* number of productive tillers per plant (24), number of seeds per panicle (147), seed L/B ratio (2.42), seed yield per plant (34.60 g), dry matter production (71.00 g) and 1000 seed weight (23.00 g). Boron plays an important part in the flowering, and seed-setting process of plants. So, improvement in days to first flowering and days to 50 per cent flowering (71 DAS and 74 DAS may be due to its role in the synthesis of ethylene and also its major role in production and retention of flower buds. Boron applied as foliar form in plants (T_4) recorded higher values for plant height. Increment in plant height, panicle length, leaf length and leaf breadth of 10.64, 11.15, 10.30 and 11.96% respectively over the control, which could be due to the increasing effect of boron on growth rate and root and shoot development (Shah *et al*., 2011). Boron is reported to be involved in maintaining cell wall structure and maintaining membrane function. It is believed to improve the strength of the membrane and cell wall with the cross-linked polymer and strengthen the

vascular bundles which hold back the invasion of pathogens and also stimulated activity of photosynthesis, chloroplast and protein synthesis (Singh *et al*., 2007). The number of tillers per plant, number of productive tillers per plant, number of seed per panicle, seed L/B ratio, seed yield per plant, dry matter production and 1000 seed weight which were 17.85, 25, 9.52, 3.30, 17.92, 18.31 and 3.04% respectively higher than the control T_0. Boron applied plants recorded higher values than control, owing to the B's involvement in reproductive growth as B improves the panicle fertility in rice (Rehman *et al*., 2012). Boron is required for pollen tube growth (Bolanos *et al*., 2004) at the time of flower pollination and increased seed set due to better starch utilization and translocation of assimilates to developing grains thereby improved the seed yield (Jehangir *et al*., 2017). The application of boron enhanced seed set by delaying abscission of flowers(Ali *et al*., 2016) and more 1000 seed weight and single plant yield reduced panicle sterility and more reproductive structure with less aborted pollen after B application. This was in conformity with the report of Phonglosa *et al*., 2018; Anand *et al*., 2020 and Laik *et al.* 2021.

In present foliar application treatment, seeds harvested from 1% boron foliar applied plants recorded higher values for the resultant seed qualities *viz.,* germination percentage (90%), speed of germination (35.40), root length (25.70 cm), shoot length (18.02 cm), seedling length (43.72 cm), seedling fresh weight (2.90 g) seedling dry weight (0.25 g), vigour index I (3934) and vigour index II (22) and lower values were recorded in control. Increased seed quality parameters by 1% Boric acid (T_4) over control could be due to synergistic role of boron in increasing the nutrient availability of calcium and sustaining it over a period as compared to their control. An enhancement in seed quality parameters was due to its better translocation and metabolism of Boron as a carrier of phosphate nutrients particularly into the seed as well as activator of enzymes like transphosphorylase, dehydrogenase and carboxylase during germination, the store of more metabolites during seed maturation and improved DNA repair mechanism by increased enzyme activity results in increased vigour of the seed. Similar results were obtained by Anand *et al*., 2020 and Shukla and Singh, 2020. Thus, in this present study, 1% boron foliar spray application had a beneficial effect on vegetative growth, yield parameters and the resultant seed quality than control.

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Table 1: Effect of foliar application on crop growth and yield attributing characters in rice

Treatment	Days to first flowering	Days to 50 per cent flowering	Plant height (cm)	Panicle length (cm)	Leaf length (cm)	Leaf breadth (cm)	Number of tillers per plant	Number of productive tillers per plant	Number of seeds per panicle	Seed L / B ratio	Seed yield per plant (g)	Dry matter production (g)	1000 seed weight (g)
T_0	**74**	**78**	**70.00**	**21.50**	**29.60**	**1.03**	**23**	**18**	133	2.34	28.40	58.00	22.30
T_1	**72**	**76**	**74.80**	**23.20**	**31.00**	**1.10**	**25**	**21**	137	2.39	32.10	63.00	22.80
T_2	**73**	**77**	**72.20**	**22.00**	**30.10**	**1.05**	**24**	**19**	134	2.36	31.20	59.80	22.50
T_3	**72**	**76**	**76.80**	**23.70**	**32.50**	**1.12**	**26**	**22**	145	2.41	33.40	66.00	22.90
T_4	**71**	**74**	**78.34**	**24.20**	**33.00**	**1.17**	**28**	**24**	147	2.42	34.60	71.00	23.00
T_5	**73**	**77**	**73.20**	**22.80**	**30.80**	**1.09**	**24**	**20**	136	2.37	31.70	61.00	22.70
Mean	**72.50**	**76.33**	**74.22**	**22.90**	**31.166**	**1.093**	**25**	**20**	138.66	2.3817	31.90	63.13	22.71
S.ED	**0.168**	**0.271**	**0.0521**	**0.052**	**0.308**	**0.027**	**0.04**	**0.037**	0.4153	0.0278	0.037	0.046	0.009
CD(0.05)	**0.376**	**0.605**	**0.1161**	**0.117**	**0.687**	**0.062**	**0.091**	**0.081**	0.9260	0.0621	0.083	0.103	0.022

Table 2: Effect of foliar application on seed quality characteristics of resultant seed in rice

Treatment	Germination %	Speed of germination	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seedling fresh weight (g seedling^{-10})	Seedling dry weight (g seedling^{-10})	Vigour index I	Vigour index II
T_0	81 (64.16)	27.20	20.39	14.55	34.94	1.64	0.18	2830	14
T_1	86 (68.03)	32.40	23.61	16.65	40.26	2.10	0.22	3462	18
T_2	83 (65.65)	28.80	21.13	15.27	36.40	1.82	0.20	3021	16
T_3	88 (69.73)	33.60	24.45	17.20	41.65	2.50	0.24	3665	21
T_4	90 (71.56)	35.40	25.70	18.02	43.72	2.90	0.25	3934	22
T_5	85 (67.26)	29.10	22.00	15.40	37.40	1.94	0.21	3179	17
Mean	85.50	30.66	22.88	16.18	39.06	2.15	0.2168	3348	18
S.ED	0.4607	0.6583	0.4899	0.054	0.053	0.059	0.030	14.26	0.374
CD(0.05)	1.004	1.4351	1.0680	0.118	0.117	0.129	0.066	31	0.817

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RESEARCH ARTICLE

Regulatory Approval Process for PRIME (Priority Medicines) in European Union

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ABSTRACT

The development of new promising medicines for unmet medical needs is always challenging from the both scientific and regulatory point of view. In concern this there, there was requirements for accelerating early access to medicines. Many patients with serious diseases have no or unsatisfactory therapeutic options and should be able to benefit from scientific advancement and cutting-edge medicines as early as possible. As a solution for this, European Medicines Agency (EMA) developed a scheme to stimulate innovation, optimize development and enable accelerated assessment of Priority Medicines (referred to as Prime) in 2014. PRIME medicines represent significant progress in their therapeutic areas. They include innovative technologies such as the first CAR – T cells therapies to be authorized, one-time potentially curative gene therapies, and rare cancer treatments. The present work encompasses listing out

the eligibility criteria, scientific elements, benefits for the patients and medicinal product developers, and evaluation of centralized marketing authorization application to an accelerated assessment prior to filing.

Keywords: European Medicines Agency, Priority Medicines, Committee for Advanced Therapies, Unmet medical requirements, Advanced Therapy Medicinal Products

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INTRODUCTION

From a scientific and regulatory standpoint, developing potential new drugs to meet unmet medical needs is difficult. Early interaction involving regulatory officials and other decision-makers in the health sector, as well as scientific assistance, is critical to assuring the data is produced to the required standards for regulatory approval and access to markets. Over the last year, the European Medicines Agency (EMA), as well as its scientific committees, have already been focusing on a number of activities aimed at access to medications that address unmet medical requirements for patients. The Committee for Medicinal Products for Human Use (CHMP) and officials from the EMA formed a group in December 2014 to look at measures to assist the novel medicinal product development that meet significant public health concerns within the current regulatory framework. As a result of the action, the plan has been developed to increase initial dialogue and regulatory support by the regulatory body in order to encourage innovation, optimize development, and expedite the review of Priority Medicines (referred to as PRIME) [1]. PRIME was launched by the EMA to enhance the support for medicines to address unmet medical requirements development. This collaborative framework focuses on increased engagement and initial contact with potential pharmaceutical developers in order to optimize programs for development and accelerate assessment so that these therapies potentially reach patients earlier [2].

Key benefits for applicants are

By European Medicines Agency's CHMP or the Committee for Advanced Therapies (CAT) rapporteur will be appointed initially. It provides constant support and assists in the development of knowledge prior to submitting to the regulatory authority for marketing authorization application (i.e., MAA)

- A kick-off discussion with the CHMP/CAT rapporteur and a comprehensive team of experts from key EMA officials, scientific committees, and working parties to
  a. give early advice on the plan for general development,
  b. As a future topic of counsel and guidance, discuss key developmental stages.
  c. strike up conversations about the suggested regulation strategy;
- Scientific guidance on overall development plans, major milestones, and critical factors, with the potential of including the other stakeholders
- Possibility of accelerated review in the marketing authorization application will be more.

The initiative enables the applicant to have an early, ongoing, and better regulatory contact with the EU regulatory network, resulting in the development of comprehensive information packages that meet MAA requirements. This will also increase applicants' awareness of existing tools that are relevant to their planning process [3].

Eligibility Criteria:

The PRIME program is intended for medicines undergoing study that have not yet received EU approval and for which the applicant wants to submit an initial MAA through the centralized procedure. PRIME's requirements for eligibility are equal to the EMA's accelerated assessment standards, and it focuses on medicines with important public health implications, particularly in terms of therapeutic innovation.

PRIME-eligible products must meet the following criteria

•Target conditions with an unmet medical need, i.e., conditions for which there is no satisfactory method of diagnosis, prevention, or treatment in the community, or for which, even if such a method exists, the medicinal product in question will provide a significant therapeutic benefit to those affected.;

•Exhibit the ability to meet an unmet medical need for community health maintenance and improvement, for example, by introducing novel therapies or improving existing ones. The evidence should back up the assertion that the product has the capacity to deliver a significant therapeutic benefit to individuals with a specific diagnosis by improving efficacy in a clinically meaningful way, such as affecting disease prevention, onset, or duration, or lowering disease morbidity or mortality

Medicines that are not eligible for PRIME;

- Medicines that don't meet an emergency medical need.
- Medicines that do not provide a significant therapeutic benefit to patients.
- Medicines that have previously been approved by the EMA[4]

Scientific Elements and Regulatory Tools for developing the development of PRIME

Before checking for the edibility for PRIME, the applicant needs to go through one of the documents released by the EMA "Draft toolbox guidance on scientific elements and regulatory tools to support quality data packages for PRIME marketing authorization applications "Scientific elements and regulatory tools for PRIME quality data packages are the document's main strengths. Throughout the process of development of medications for early access, applicants confront obstacles in meeting quality and manufacturing development as well as data requirements. Using a 'toolbox approach,' this document summarises scientific elements and regulatory tools available in the existing EU regulatory framework to contribute to the development and completion of Module 3 quality data packages in the preparation of marketing authorization applications (MAA) for designated PRIME medicinal products. On November 26, 2018, the Workshop on Supporting Quality Development in Early Access Approaches with Stakeholders, co-hosted by PRIME and the US Food and Drug Administration (FDA), aimed to identify scientific and regulatory solutions to challenges faced by PRIME applicants in completing Module 3 data requirements in time for the MAA.

Important tools as per the document

1. Scientific Tools
    - General scientific tools
    - scientific tools related to process validation
    - scientific tools related to contrail strategy
    - GMP compliance
    - scientific tools related to stability
    - scientific tools related to comparability
2. Regulatory tools
    - Accelerated assessment
    - Conditional marketing authorization
    - Other tools
    - Post approval change management protocol
    - Post authorization measures[5]

Criteria for applying for PRIME eligibility in the stage of development

A request to join the PRIME system can be made by any sponsor during the stage of development where an exploratory clinical study is being conducted, based on preliminary clinical results in patients revealing the medicinal product's promising effectiveness and ability to treat a significant unmet medical need (proof of concept). In exceptional circumstances, applicants from the academic sector and micro, small, and medium-sized businesses (SMEs) can file an eligibility request at an early stage of the research., if

•Non-clinical findings in a set of architecture are convincing and indicate possible action early on (proof of principle)and
•first-in-man studies have shown that adequate exposure is required for the desired tolerability and pharmacotherapeutic effects
SMEs must register with the Agency's SME office before filing an eligibility request. Applicants who feel they meet the criteria for the academic sector should contact the Agency before filing an eligibility request. If a product's development is already well underway (For example, a key study is now ongoing, and scientific guidance has been sought), The rest of the development and post-authorization tasks for which PRIME would be useful must be detailed in the application.

Steps involved in the application
Following the requirements below, the applicant should send an eligibility request to PRIME via Eudralink2 to prime@ema.europa.eu.
•Request form for Pre-submission
•Template for the applicant's justification
• Literature references cited in the justification
Any Human Medicinal Product that requires marketing permission must submit a pre-submission request form. Applicants should first choose the sort of medicine they would like to market, which should be a Human Medicinal Product. Following that, a component of sector PRIME selects the date of the request. There is no charge for submitting a request to join the PRIME program.
Template for the applicant's justification – (This document must be supplied in Microsoft Word format.), The product information should be present in this template
The following informationis there as per the template Literature references cited in the justification – (This has to be included in a zip file)In the Applicant's justification template what are the citation and references used to give the information about the product should be mentioned [6], [7],[8].

Stages of Evaluation of eligible PRIME product
The SAWP will assess PRIME eligibility requests, in the case of ATMP (Advanced Therapy Medicinal Products), the CAT and the CHMP will be responsible for implementing recommendations. EMA should receive an electronic request for PRIME support from the applicant., as well as a reason and then a summary of the data available After receiving the request, the procedure will be allocated to one SAWP reviewer and one EMA scientific officer., which will begin according to the specified timelines. On day one SAWP 1 meeting will start, it is the starting of the procedure. On day 30 SAWP 2 meeting, the SAWP plenary will discuss the eligibility and recommendations. The final suggestion of the CHMP will be adopted during the plenary meeting on day 40. Requests for ATMPs will be assigned to one CAT reviewer and disseminated to the CAT for evaluation and recommendation following the SAWP, prior to finalization and acceptance by CHMP. The applicant will receive the conclusion, including the reasons for the CHMP's decision, through EMA. If additional evidence or data is determined to support eligibility for the plan, the applicant may resubmit a fresh request. To examine such a new request, a new SAWP reviewer and an EMA scientific officer will be assigned. In order to validate PRIME eligibility, the same method will be used to assess the applicant's justification of clinical proof of concept for a product that joined the system in the early phases of development. Such requests shall be considered by the SAWP reviewer and EMA scientific officer assigned at the time of the first request, wherever practicable. The CHMP's conclusions will be made available to the general public. The CHMP monthly report will include a summary of the number of recommendations adopted following each CHMP meeting, including:
•the product type (chemical, biological or advanced therapy),
• the indication of intension,
•the sort of information that backs up the eligibility request and,
•the sort of candidate (SMEs, applicants from the academic sector or others)

Approval of Eligible PRIME medicinal product

Confirmation of Centralized procedure eligibility

The applicant will get confirmation of eligibility for the centralized procedure the same month that the CHMP recommends entry to the program, based on the information provided in the PRIME eligibility request form.

Rapporteurs Appointment

The appointment of a CHMP rapporteur can also commence. (Unless the applicant is an SME or an academic who has sought to join the plan is based on data that proves the proof of principle). For advanced-therapy medical products, a CAT rapporteur and a CHMP coordinator will be established (ATMP). A month is required for the appointment process after the CHMP meeting. The applicant will be notified about the outcome and confirmation of PRIME eligibility. The applicant must submit a letter of intent before the CHMP co-rapporteur and PRAC rapporteur may be appointed. (6-7 months before MAA submission). The selection of the rapporteur for SMEs or academic applicants who are accepted into the plan based on data demonstrating proof of principle will take place after they've gathered information demonstrating eligibility at the proof-of-concept stage. As the product development progresses, the applicant will be expected to supply pertinent data and justification.

EMA dedicated contact point

During the development, the applicant will have a specific EMA contact person who will coordinate the support provided throughout the plan. The eligibility outcome letter will include the applicant's name and information. The EMA will continue to aid development by providing regulatory guidance and, Raising awareness of the use of regulatory/legislative mechanisms where applicable. (For example, conditional marketing authorization and marketing authorization in unusual circumstances) additional measures to improve patient access to care.

Kick-off meeting

As soon as possible after entering the program, the EMA contact point will work with the applicant to organize a kick-off meeting with the CHMP rapporteur and other relevant experts from the EU network.(In the case of ATMP, in particular from PDCO, COMP, PRAC, SAWP, and CAT and necessary EMA personnel.).The goal of this event is to assist the applicant's early engagement with the EMA and the multidisciplinary evaluation experts' group. Whereas the participants are unlikely to interactin in-depth discussions about the list of subjects related to scientific and technical issues, the goal of the kick-off meeting is to agree on the next steps for addressing any identified issues or identifying potential new issues. approach and the product's development plan will be presented during the meetings. Through applicable regulatory procedures, the applicant will obtain advice on interactions. (For example, sufficient timeframes for submitting a request for scientific advice, or a paediatric inquiry plan).

Scientific advice

Scientific advice is given in the framework of scientific advice processes, where the applicant can get the advice comprehensively on the plan of development as well as on important concerns or particular important subjects for the MAA. In accordance with existing practice, each operation will be assigned two SAWP coordinators. Each iterative scientific advice request will be assigned to one of these SAWP coordinators. This is expected to make information exchange to life-cycle management from development and the production of SAWP/CHMP guidance straightforward. From the same delegation as both the CHMP rapporteur and the SAWP coordinator from SAWP will be appointed. wherever practicable. Different committees (e.g., CAT, COMP, PDCO) will be engaged in the final advisory letter development if needed. Applicants will also be encouraged to obtain EMA/HTA advice at the same time. If the applicant is an SME or an academic, on a case-by-case basis, they may be eligible for a cost reduction on scientific advice requests pertaining to a PRIME product. This is something that can be addressed with the EMA's dedicated contact point.

Regular updates

The Applicant shall keep the EMA dedicated contact point informed of any processes filed to the Agency following the kick-off meeting. If the applicant discovers a subject that has to be discussed with regulators further, they must

approach the EMA and it provides guidance for further proceedings. When necessary, the Agency can facilitate meetings between the applicant and the CHMP/CAT Rapporteur (e.g., ad hoc teleconferences) in order to settle tiny concerns or give updates on the advancement of the applicant. The European Medicines Agency's Guidance on Interactions in the Context of PRIME provides more information.

Accelerated assessment

The accelerated assessment procedure is beneficial to medicinal goods that have been awarded PRIME assistance, However, this will need to be legally confirmed for marketing authorization submission before 2-3 months. [9], [10], [11].

CONCLUSION

Many patients with serious health complications have no or are unsatisfied with therapeutic actions and need to be benefited from scientific advancements in medicines. Such medicines for an unmet need are eligible for PRIME, through which promising medicines can reach the patients at the earliest via accelerated assessment of marketing authorization application. PRIME is a kind of designating process that involves multiple committees for robust assessment. Weak pharmacological rationale insufficient non-clinical evidence on the mechanism of action, limited relevance of animal models presented, insufficient Pharmacokinetics data to support the expected clinical outcome, trial design issues, and sample issues could be the main reason for denial of the PRIME application.

ACKNOWLEDGEMENT

NIL

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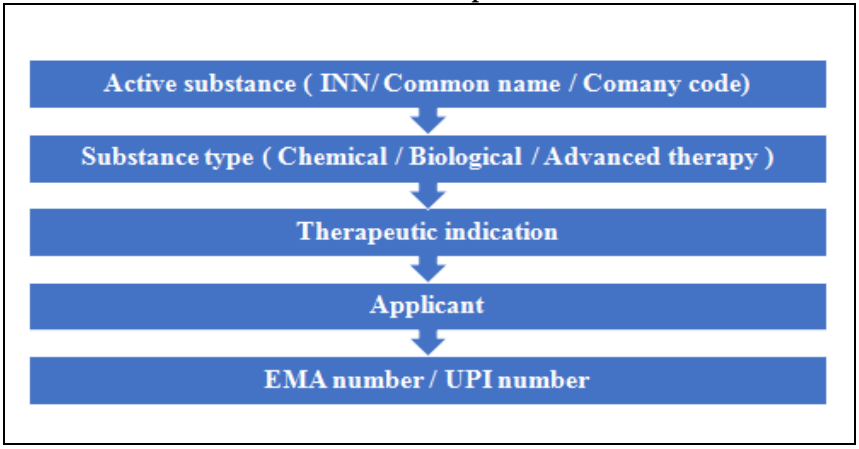
Table 1: Information mentioned in the template



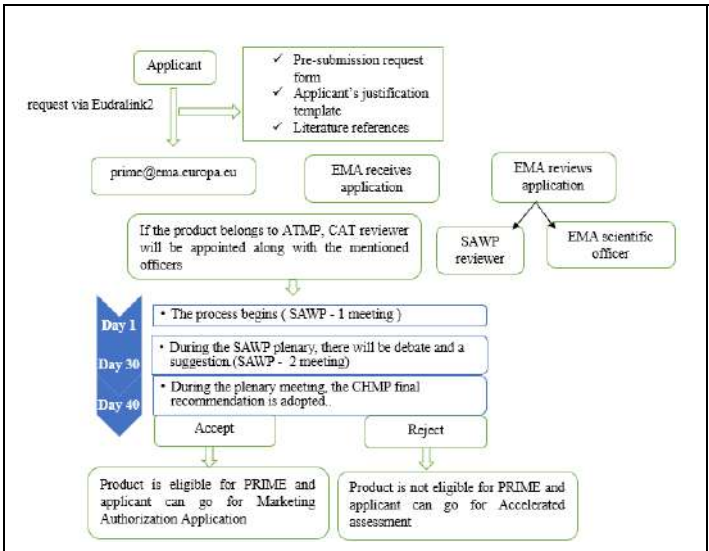


Figure 1: Flow process of Medicinal Products for PRIME designation

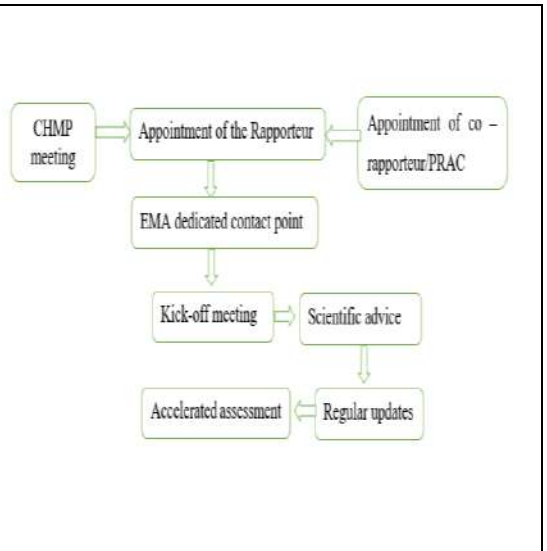


Figure 2: Final approval process for eligible PRIME

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RESEARCH ARTICLE

Effect of Sequential Application of Newer Herbicides for Management of Weeds in Irrigated Blackgram

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ABSTRACT

Field experiment was conducted at farmer's field at Gudamalai village, Gangavalli taluk, Salem district during *Rabi* season 2019 to evaluate the performance of different pre-emergence and early post emergence herbicides on weeds and productivity of blackgram under irrigated conditions. The experiment was laid out in randomized block design with three replications. The experiment consists of ten treatments T_1- Pendimethalin @ 1.0 kg ha^{-1} on 3 DAS, T_2- PE Pendimethalin @ 1.0 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g/ha (Odyssey), T_3- PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS, T_4- PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + HW on 25 DAS, T_5- PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g/ha (Odyssey), T_6- PE Oxadiazone @ 250 g ha^{-1} on 3 DAS + EPOE Imazethapyr @ 70 g ha^{-1} (2 -3 leaf stage of weeds),T_7- PE Oxadiazone @ 250 g ha^{-1} on 3 DAS + EPOE Imazethapyr @ 70 g ha^{-1} + Quizalofop-ethyl @ 50g/ha(2 -3 leaf stage of weeds, T_8- EPOE Imazethapyr @ 70 g ha^{-1} + Quizalofop-ethyl @ 50g/ha (2 -3 leaf stage of weeds) – Tank mix, T_9- Hand Weeding twice @ 20 and 40 DAS, T_{10}- Unweeded check. Among the different weed control treatments imposed hand weeding twice at 20 and 40 DAS (T_9) registered the maximum growth (Plant height , leaf area index and Dry matter production) yield attributes (Number of pods per plant, Number of seeds per pod) and Yield (Grain yield kg ha^{-1} ,Haulm yield kg ha^{-1}) of irrigated blackgram due to reduced weed growth, weed dry matter production and reduction in nutrient depletion by weeds and increased nutrient uptake by crop and this was followed by pre emergence application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g/ha (Odyssey) (T_5).Application of pre-emergence Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr +

imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) registered highest net income and benefit cost ratio compare to other treatments. The lowest growth and yield and yield attributes and economics was recorded under unweeded check (T_{10}).

Keywords: Blackgram, Newer herbicides, Growth, Productivity and Economics

INTRODUCTION

Pulses are the major source of supplementary protein to daily diets and starchy food for a predominantly vegetarian population. Pulses are cheaper than meat and therefore often regarded as poor man's meat. Pulses contain on an average of 20-25 percent protein (Sooraj Chandra Pankaj *et al.,* 2017). Pulses are wonderful gift of nature with the unique ability of biological nitrogen fixation, deep root system, mobilization of insoluble soil nutrients and bringing qualitative changes in soil properties which make them known as soil fertility restorers (Deepak Kumar *et al.,* 2018). The total world acreage under pulses is about 85.40 million hectares with a production of 87.40 million tonnes at 1023 kg ha^{-1} yield level. In India, pulses are being cultivated under an area of 29.36 million hectares with the production of 24.51 million tonnes and productivity of 835 kg ha^{-1}. The total area under pulses in Tamil Nadu is 8.16 lakh hectares with a production of 5.72 lakh tonnes. (DES, Ministry of Agriculture &FW (DAC & FW), Govt of India, (2018).). Their productivity of pulses can be doubled by improved cultivars and by modern production technologies Sooraj Chandra Pankaj *et al.,* 2017).

Blackgram (*Vigna mungo* (L.)) is one of the most important pulse crops, which can be grown in tropical and subtropical regions. It is native of India and originated from *Phaseolus sublobatus* a wild plant. (Deepak Kumar *et al*. 2018). Blackgram is very nutritious as it contains a high level of carbohydrate (60g/100g), protein (20-25 g/100 g), phosphorus (385 mg/100 g), calcium (145 mg/100 g) and iron (7.8 mg/100 g). It is useful in mitigating elevated cholesterol levels. The total world acreage under blackgram is about 23.48 million hectares with a production of 15.43 million tonnes at 653.07 kg ha^{-1} yield level. The total area under blackgram in India is around 4.47 million hectares with a production of 2.83 million tonnes and productivity of 632 kg ha^{-1}. In Tamil Nadu, blackgram is cultivated in 4.30 lakh hectares with the production of 2.74 lakh tonnes and an average productivity of 637 kg ha^{-1} (Indiastat, 2019). In blackgram there are various factors responsible for the reduction in growth and yield. The main constraint in blackgram production is weed infestation during its growth period which inflicts heavy losses on the crop yield by competing for essential growth factors. Weed management at the early stages of crop growth is essential, leading to severe competition between the crop and weeds. (Sukumar *et al*. 2018). Weeds compete for water, nutrient and space and cause up to 45% yield loss in Blackgram (Yadav *et al.,* 2015). An initial period of crop weed competition of 20-40 days is very critical and weed competition reduce blackgram yield to the extent of 87 percent. (Arvind verma *et al*. 2017). Weed management is an important key factor for enhancing the productivity of irrigated blackgram since the crop is not very good competitor against weeds (Choudhary*et al.* 2012). Hand weeding during critical crop growth stages is not possible due to increased cost and scarcity of human labour. Application of pre emergence herbicide lasts long only a very short period of the crop growth period. After the loss of herbicide concentration weed seed bank start to emerge and compete with crops for natural resources. Delayed removal of weeds is not as effective in controlling weeds. Application of early post emergence herbicide controls late emerging weeds and obtains higher yields against the timely removal of weeds (Pratap Singh *et al*. 2016). Under such circumstances, pre and early post emergence herbicides applied in sequence or combination will controls the weeds very effectively. Keeping these points in view, the present study was undertaken to find out the effect of pre and early post emergence herbicides and hand weeding practices on growth and yield ,economics of irrigated blackgram.

MATERIALS AND METHODS

The experiment was conducted at Gudamalai village, Gangavalli Taluk, Salem District, Tamil Nadu. The experimental farm is at 11˚46 N and 78˚59 E an altitude of 230 meters above MSL.The mean annual rainfall of the place is 952.2 mm. The mean maximum and minimum temperature prevailed during the cropping period was 34.9˚C and 26.3˚C respectively. The mean relative humidity ranges from 60.5 to 77.4 percent. The texture of the experimental field soil was sandy clay loam with neutral pH and low, medium and high in available nitrogen, phosphorus and potassium respectively. The popular variety Vamban(Bg) 5 was chosen for the study. The experiment was laid out in Randomized Block Design (RBD) with three replications and ten treatments. The treatment schedule were as follows : T_1- PE Pendimethalin @ 1.0 kg ha^{-1} on 3 DAS, T_2 - PE Pendimethalin @ 1.0 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g/ha (Odyssey), T_3- PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS, T_4 - PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + HW on 25 DAS, T_5 - PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g/ha (Odyssey), T_6 - PE Oxadiazone @ 250 g ha^{-1} on 3 DAS + EPOE Imazethapyr @ 70 g ha^{-1} (2 -3 leaf stage of weeds), T_7 - PE Oxadiazone @ 250 g ha^{-1} on 3 DAS + EPOE Imazethapyr @ 70 g ha^{-1} + Quizalofop-ethyl @ 50g/ha(2 -3 leaf stage of weeds), T_8 - EPOE Imazethapyr @ 70 g ha^{-1} + Quizalofop-ethyl @ 50g/ha(2 -3 leaf stage of weeds) – Tank mix, T_9 - Hand Weeding twice @ 20 and 40 DAS, T_{10} - Unweeded check. Good and viable seeds are selected for sowing.The treated seeds were sown at 30 cm between rows and 10 cm between seed to seed to maintain plant population of 3,33,333 plants ha^{-1}.The recommended dose of 25:50:25 kg of NPK + 40 kg 0f S ha^{-1} was applied as basal application. Thepre-emergence herbicides and early post emergence herbicides were applied on 3 DAS and 12 to 15 DAS as per the treatment schedule. Hand weeding was done on 20 and 40 DAS as per treatment schedule. Observations were recorded using quadrant (0.5 × 0.5 m). The biometric observations on growth and yield characters were recorded from five randomly selected plants. Plant growth parameters like plant height, dry matter production and leaf area index and yield parameters like number of pods plant^{-1}, number of seeds pod^{-1}, pod length, 100 grain weight, grain yield and haulm yield were recorded to assess the crop productivity. Theeconomic parameters like cost of cultivation, gross income, net income and benefit cost ratio (BCR) were also estimated to assess the influence of different weed control methods.

RESULTS AND DISCUSSION

Effect of weed control methods on growth and yield

Effect on growth attributes (Table:1)

Among the weed control treatments, the plant height, leaf area index and DMP significantly influenced by various treatments. On 30 and 45 DAS higher plant height (20.42,36.00 cm), leaf area index (4.95 on flowering),DMP (872, 2284 kgha^{-1}) was registered in hand weeding twice on 20 and 40 DAS (T_9) and this was followed by the herbicidal treatment PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) with a plant height(18.89 and 33.48 cm) leaf area index (4.52 on flowering stage), DMP (840, 1928 kgha^{-1}) on 30 and 45 DAS respectively. A weed free environment until the critical period of the crop growth by hand weeding facilitated good growth of the crop. Improved nutrient uptake and vigour due to elimination of weed competition right from the beginning of the crop might have contributed to favorable growth components, higher nutrient uptake and consequently higher plant height, LAI and DMP in hand weeding twice treatment. The superiority of hand weeding practice at 20 and 40 DAS may be attributed to better weed control, least nutrient accumulation by weeds and better aeration of the crop. Similar findings were revealed by Manoj Kumar Sandil *et al.,* (2015).Similarly, in herbicide application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) applied plots caused reduction in weed density, which might have improved the availability of resources viz., space, soil, moisture, light and nutrients to the irrigated blackgram, thus resulted in higher growth parameters.The increase in growth attributes under these treatments might be attributed due to the reduction in weed competitiveness with the crop, which ultimately favored better environment for growth and development of crop. Similar results are reported by Harithavarthini *et al.,* (2016).The least plant height, leaf area index, DMPwas recorded under unweeded check (T_{10}).

Effect on yield attributes

All the treatments had a pronounced effect on the yield attributes of irrigated blackgram. Among the weed control treatments showed a noticeable influence on the number of pods plant^{-1}on harvest (36.85) and pod length (5.5 cm) was recorded in hand weeding twice on 20 and 40 DAS (T_9) and this was followed by the herbicidal treatment PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) with the number of pods plant^{-1} of 33.84 and pod length (5.1 cm) on harvest respectively.. Among the treatments, hand weeding twice on 20 and 40 DAS (T_9) provided a perfect weed free environment throughout the critical period of crop growth and offered the highest value of yield components in the crop. This might be attributed to reduced crop weed competition in the critical stages which helped in synchronization of their production by increasing the number of pods plant^{-1}, number of seeds pod^{-1} and pod length. These results were in agreement with the findings of Chhodavadia *et al.,* (2013), Manoj Kumar Sandil *et al.,* (2015) and Khot *et al.,* (2016).Among the various chemical weed control methods, Pre emergence application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) recorded the highest values of yield components and the herbicide treatment pre emergence application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + HW on 25 DAS (T_4) was next in the order. It could be attributed to significantly lower weed population, dry matter accumulation and higher weed control efficiency of weeds and also due to weed free environment provided by this treatment which might have increased the translocation of assimilates from source to sink and hence the yield attributes viz., number of pods plant^{-1}, number of seeds pod^{-1} and pod length increase in this treatment. These results were in agreement with the findings of Patel *et al.,* (2015). The lowest yield attributes were recorded underunweeded check (T_{10}). Severe weed competition exerted by weeds for the available resources throughout the crop growth might have reduced the yield components.

Effect on yield

Among the different treatments hand weeding twice @ 20 and 40 DAS (T_9) registered the maximum grain and haulm yield and it was 51.09 and 40.91 percent higher over unweeded check (T_{10}). The highest grain yield (912 kg ha^{-1}) and haulm yield (1459 Kgha^{-1})was recorded in hand weeding twice on 20 and 40 DAS (T_9) and the application of herbicidal treatment PE Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) was next best and recorded 54.37 and 44.95 percent of grain yield (857 Kgha^{-1}) and haulm yield (1328Kgha^{-1}) over unweeded check (T_{10}). This would be due to effective control of weeds which reduced the crop weed competition and increased yield of blackgram. These results were in agreement with the findings of Khot *et al.,* (2016).This would be due to effective control of weeds which reduced the crop weed competition and increased yield of blackgram.The unweeded check showed the real depiction of the aggressive nature of weeds on the growth of irrigated balckgram. The lowest grain(466 Kgha^{-1}) and haulm yield (597 Kgha^{-1})were recorded in unweeded check(T_{10}). This might be due to the severe competition between crop and weed for different resources.

Economics

Economic efficiency and viability of crop cultivation are the main criteria for successful crop production. In general, higher crop productivity resulted in better economic parameters like net income and benefit cost ratio. Among the treatments imposed, hand weeding twice at 20 and 40 DAS (T_9) registered the highest gross income of ₹57273 ha^{-1}. But in terms of net income and BCR, pre emergence application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) registered the highest net income of ₹39491ha^{-1} and BCR of 2.40.This might be due to the less cost of cultivation. The above result was in line with the findings of Sharma *et al.,*(2014).However, the pre emergence application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + HW on 25 DAS (T_4) closely followed the above treatment with regard to net return with marginal reduction when compared to the treatment (T_5). Hand weeding twice 20 and 40 DAS (T_9) recorded the lower BCR due to the higher cost of cultivation. In hand weeding, the labour cost escalates the cost of cultivation due to the high wages of manual labour. The lowest gross income, net income and benefit cost ratio invested were recorded in weedy check an account of the severe reduction in grain yield due to weed competition throughout the cropping period. Similar results were also reported by Naidu *et al.,* (2011).

CONCLUSION

In the light of above said facts, it can be concluded that adoption of hand weeding twice at 20 and 40 DAS (T_9) recorded the higher growth attributes, yield attributes, grain yield and haulm yield and economics in irrigated blackgram and it was followed by pre emergence application of Pendimethalin (38.7%) @ 0.75 kg ha^{-1} on 3 DAS + EPOE Imazethapyr + imazamox (RM) 70 g ha^{-1} (Odyssey) (T_5) holds promise as an agronomically sound, ecologically safe and economically viable technology for enhancing the yield of irrigated blackgram.

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Table 1. Influence of weed management practices on growth

Treatment	Plant Height (cm)		Leaf Area Index	Dry matter production at harvest (kg ha^{-1})	
	30 DAS	45 DAS		30 DAS	45 DAS
T1	12.68	24.28	2.92	664	1531
T2	15.82	28.59	3.69	773	1774
T3	11.20	21.79	2.51	627	1448
T4	17.38	31.01	4.1	809	1852
T5	18.89	33.48	4.52	840	1928
T6	14.98	27.38	3.43	726	1668
T7	15.41	27.95	3.57	749	1722
T8	13.20	24.87	3.03	687	1587
T9	20.42	36.00	4.95	872	2284
T10	9.62	19.27	2.10	585	1351
S.Ed	**0.26**	**0.55**	**0.17**	**15.66**	**39.34**
CD(P= 0.05)	**0.52**	**1.12**	**0.34**	**31.64**	**79.47**

Table 2. Influence of weed management practices on yield parameters and yield

Treatment	No. of pods Plant^{-1}	No of seeds pod^{-1}	Pod length(cm)	Test weight (g)	Grain yield(kg ha^{-1})	Haulm yield(kg ha^{-1})
T1	23.38	5.72	3.6	3.8	621	835
T2	29.11	6.08	4.3	4.3	758	1057
T3	20.91	5.64	3.2	3.6	540	693
T4	31.47	6.17	4.7	4.5	805	1182
T5	33.84	6.29	5.1	4.7	857	1328
T6	27.03	5.97	4.1	4.1	700	992
T7	28.01	6.03	4.2	4.2	733	1026
T8	24.58	5.88	3.7	3.9	652	880
T9	36.25	6.40	5.5	4.9	912	1459
T10	18.33	5.30	2.9	3.5	466	597
S.Ed	**1.12**	**-**	**0.17**	**-**	**19**	**31**
CD(P= 0.05)	**2.27**	**NS**	**0.35**	**NS**	**38**	**62.43**

Table.3: Economics of irrigated blackgram as influenced by weed management practices

Treatments	Cost of cultivation(Rs ha^{-1})	Gross income (Rs ha^{-1})	Net income (Rs ha^{-1})	Benefit cost ratio (BCR)
T_1	22212	38919	16707	1.7
T_2	23449	47524	24075	2.1
T_3	22070	38826	16256	1.5
T_4	23080	50501	27421	2.2
T_5	23307	53798	39491	2.4
T_6	22507	43896	21389	1.9
T_7	22684	45959	23275	2.0
T_8	22462	40864	18402	1.8
T_9	27201	57273	30072	2.1
T_{10}	21141	29190	8049	1.3

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RESEARCH ARTICLE

Student Attentiveness Analysis System for Online Learning

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ABSTRACT

Since the coronavirus pandemic unexpectedly and forcibly moved classroom activities to a totally remote format, there is a critical need for progress in the online educational system. Additionally, as online education is the wave of the future and requires enhanced infrastructure, learning and teaching process. The main issue with the present video is analysis of student involvement is done using a call-based online classroom system. Teachers frequently worry about how well their pupils will understand new information. Such analysis was unintentionally performed in the offline mode, but it is challenging in an online setting. This study introduces an autonomous method for monitoring student emotions to gauge their level of participation in class. This is accomplished by taking a screenshot of the students' video stream and sending the faces that are discovered to an emotion detection mode. The suggested architecture's emotion detection model was created by optimising the VGG16 pre-trained image classifier model. The average student engagement index is then determined. We then have also tested the model using the face recognition system for the result. We saw significant performance setting dependability of the suggested system's employment in real-time offering this research potential reach.

Keywords: Emotion detection, CNN, VGG16, Education, Transfer learning, Engagement.

INTRODUCTION

Social software technology has advanced in the realm of education with the introduction of the internet. Many scholars have focused on intelligent tools to improve the educational system, especially as digitalization has increased.

Educational Data Mining (EDM) is a popular subject of study that examines how data science might be used to educational data [1,2, 28]. The authors of [2] explore how educational data can be used for a diversity of rationales, including predicting student performance, recommending courses, predicting early dropout, and more. In this study, we look at a similar application of employing computer vision to analyse students' engagement in an online video conferencing-based classroom system. Interest in e-learning is increasing, especially during pandemics, and it appears to be increasing even more in the future. There are no essential tracking systems accessible for educational institutions to track student engagement during lectures and sessions, leaving professors unable to monitor their students' development. As a result, the application discussed in this paper is now more important than ever. By observing how students responded to what they were learning in a classroom environment on campus, teachers could receive continuous response on their instruction. Such feedback is frequently helpful in gauging students' attitudes toward particular topics in class and enabling teachers to take necessary action. For instance, if the teacher detects that the class is having difficulty with a certain subject, he or she can recognise and review it. However, such appears to be lacking in online classroom systems. Raes *et al.* explore the challenges that teachers experience in engaging students in a remote learning environment vs a face-to-face learning setting in [3]. Both students and professors in Weitze's study [4] stated that remote pupils learned less.

The goal of developing a machine learning model by utilising knowledge from existing models in the same domain is loosely defined as transfer learning [17]. When there is less data for the current tasks, such approaches of fine-tuning pre-trained deep learning models are particularly advantageous [18]. Transfer learning can be aided by these three strategies. Before any further learning, there is the initial performance that can be achieved with the transferred knowledge. The second aspect is the time it takes to complete learning using transferred knowledge versus learning entirely from start. Third, compared to the final level without transfer, the final performance level achieved in the target task with transferred information [17]. Fig.1 depicts the level of precision. Pre-trained neural network models like Res Net, Mobile Net, VGG, Inception, and others have been shown to perform better than conventional methods for a number of tasks [19]. We employed the VGG16 pre-trained neural network model in this study because it was trained on 3.31 million photos of person identity. The nature of this model is therefore best suited to our objective of emotion recognition [20].

LITERATURE SURVEY

Facial expressions are the most frequent way for living things, including humans, to indicate mood or sentiments. Since facial expression analysis technology is valuable in so many different domains, such as lie detection, medicine, and robotics [5-7], it has experienced a lot of improvement. In a recent study using the Facial Recognition Technology (FERET) dataset, Sajid *et al*. found that the influence of face asymmetry is a marker of age estimate [8]. Since the eighteenth century, Ekman *et al*. [9] have identified seven moods or expressions, independent of culture, tribe, or nationality, in which a person evolves. Anger, despair, anxiety, disgust, temptation, happiness, and astonishment were among them. Mehendale [10] outlined a technique for improving facial emotion detection performance by utilising single-level CNN and creative backdrop removal techniques in a recent article on Facial Emotion Recognition using Convolutional neural networks (FERC). McDuff *et al* [11] at MSR created procedures to detect three characteristics of human emotion: valence (testing the positiveness or negativeness), arousal (emotion degree), and involvement level. These algorithms take data from hardware sensors such as microphones, web cameras, GPS, and so on. They also used interaction data such visited online URLs, documents opened, programmes used, emails sent and received, and calendar appointments. Most of the time, the inferences were used to help people think back on the previous week's activities and reflect on their feelings. Dewan *et al*. used computer vision to investigate remote audience participation. They simply measured two scenarios, bored and engaged, and used the OpenCV library[16] and computer vision to present the result of the audience's participation. Video-based learning has become popular due to the increase in businesses providing services like tutoring and test preparation that are video-based for a wide variety of reasons[12], the most notable of which is the ability to have simultaneous voice and visual communications. According to theories, two subsystems are involved [13,14]. The first is visual object processing, and the second is verbal object processing. They occur in our brains individually and can only process a certain amount of information [13], which causes kids to become distracted. Researchers have suggested several methods for better comprehending how students learn from

movies using video learning analytics. For example, Kim *et al*. [15] examined learners' in-video dropout rates and interaction peaks in online lecture videos by analysing their video-watching patterns (pausing, playing, replaying, and quitting). However, there is currently little study on how students interact with video lectures, making it difficult for instructors to assess the efficiency of the learning design, particularly at the fine grain level.

Many researchers have lately used transfer learning in conjunction with artificial intelligence and machine learning models. Hazarika *et al*. proposed using transfer learning and natural language processing to recognize the state of emotion in a conversational text [23]. Current computer vision systems have benefited greatly from transfer learning. Through transfer learning, Knetsch et colleagues used computer vision and deep-learning algorithms to analyse drone-acquired forest pictures for invasive species . He also claims that using transfer learning in his study boosted accuracy by 2.7 percent [24]. The literature on computer vision has described a range of methods for using transfer learning for a variety of applications. Additionally, researchers have used this method to recognise emotions. But there is limited research on integrating emotion recognition in teaching-learning environments to enhance online learning systems, which is why this study was conducted.

DATASET

The main objective of this project was to create a model that could recognize students' facial expressions while they were in class. A human being is capable of displaying a variety of facial emotions. However, we took into account those facial expressions that are critical to a student's learning process. Additionally, this was chosen in accordance with what teachers believe to be the most crucial elements of in-class learning. According to psychological research, positive emotions such as concentration, happiness, and satisfaction promote students' learning interests, motivation, and cognitive activities, whereas negative emotions such as boredom, sadness, anxiety, and others can have a negative impact on students' commitment and patience [21]. We looked at four different types of emotions in this paper: confused, tired, pleased, and neutral. The facial datasets for each class were scraped from publicly available Google pictures. Web scraping is the practice of extracting content and data from a website using bots. Web scraping is done with a variety of bots for purposes such as identifying unique HTML site structures and retrieving data. Content transformation, data scraping, and data extraction from API [26]. Scraping publicly accessible Google pictures from the internet is one of the most effective techniques to get data for the model. We scraped the image using Python and the Firefox Web Driver. This method has the advantage of retrieving thousands of photos "from the wild." And we can use the keywords in the query to automatically label the images [25]. For scraping, we used the Selenium and Beautiful Soup libraries for scraping purpose. The data has been made public in order to broaden the scope of this study and potentially future efforts to improve the current system.

METHODOLOGY AND IMPLEMENTATION

The suggested project pipeline's first module is to take a grid-format image of a live online classroom screen. This image is fed into a face detection model, which recognizes individual faces and separates them as independent image files. Open CV is used to create the face detection model. Individually identified facial images are supplied to the emotion detection algorithm further in the pipeline. The VGG16 pre-trained model was used in this study, and it was fine-tuned for our dataset. Simonyan and Zisserman of the University of Oxford suggested VGG16 as a convolutional neural network model in their paper "Very Deep Convolutional Networks for Large-Scale Image Recognition" [27]. In Image Net, which contains over 14 million photos belonging to 1000 classes, the model achieves 92 percent top-5 test accuracy. It raises the depth of the architecture with very small (3 3) convolution filters, demonstrating that raising the depth to 16–19 weight layers achieves a considerable improvement over prior-art designs. This model took weeks to train and was powered by NVIDIA Titan Black GPUs. Using a larger dataset is one of the most effective techniques to avoid over fitting. However, the data scraping (which was done) resulted in junk data containing irrelevant photographs. As a result, we examined image augmentation to expand the dataset by transforming the scraped relevant dataset in several ways. Rotation, width shift, height shift, shear, zoom, and horizontal flip are some of the

picture augmentation techniques used in this study. Finally, the emotion detector model finds individual emotions and based on that the class emotion index is calculated using the following formula.

RESULTS AND DISCUSSION

FACE EXTRACTION MODEL

The suggested pipeline begins with a face detection module, which was addressed in length in the preceding section. The result produced when a grid of faces (as expected in an online classroom environment) is provided to the face-detection module is illustrated in **Fig. 2**

Performance of Emotion Detection Model

An emotion detection model is also given these images. **Fig. 3.** displays the training and validation accuracy for the 10 epoch proposed fin modified model. It was time to stop training to prevent overfitting after the fourth epoch, when the training accuracy appeared to nearly flatten (parallel to the x-axis) at an accuracy of 74 percent (approx). However, the validation accuracy kept getting better. We investigated if there was any additional movement by running the model for 15 epochs. **Fig. 4** displays the training and validation accuracy for the same model after 15 iterations. The performance in this case was not especially fluid. However, it was demonstrated that both the training and validation accuracy were increasing. To further understand, we ran the model again with the same settings. This time, the findings were a little more consistent, with the validation and training curves flattening out at an average-adjusted accuracy of 77.5 percent.

Emotion Detection Model Output

Later we tried testing the model, the results obtained correctly identified the emotion classes namely Neutral, Sad, Happy and Confused with respect to the facial angles. The results obtained were 77.5 percent accurate and the output of the model exhibiting the emotion is shown in the **Fig. 5.**

CONCLUSION AND FUTURE WORK

This study's architecture is designed to examine how well students are participating in online classes that use video. The architecture starts with identifying the faces of the students in their video stream. After being cropped, each identified face is sent to the emotion detection model separately. The model for emotion detection is a pre-trained, fine-tuned transfer learning model on VGG16. Reliable validation accuracy for the work was found to be 77.5 percent. The amount of students who display each of these independently recognised emotions is then used to create an emotion index. The model is also tested with the emotion detection model, which gave the accurate outputs based on the facial symmetry. Future research will focus on improving accuracy and real-time testing of the concept as we expand our current focus.

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This is to acknowledge that during an online class, we evaluated our proposed system leveraging the realistic faces of Mr. Karan K. V., co-author of this paper, and student's faces to observe their emotions. We are grateful to our students for helping us successfully experiment the proposed system.

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Table 1: Number of images training and validation images in each class in dataset

Class Name	Happy	Confused	Neutral	Sad
#training images	126	228	161	168
#validation images	18	18	18	18

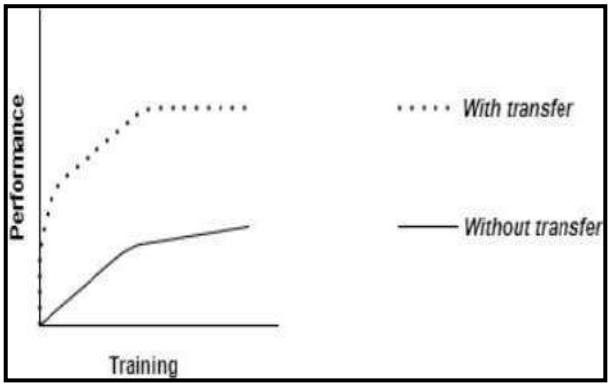

Fig 1. General observed performance of model with and without transfer learning

Fig. 2. Face to Face detection in online class

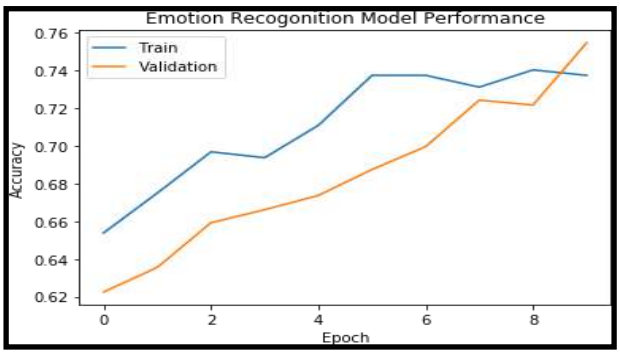

Fig. 3. Training and validation performance on 10 epochs

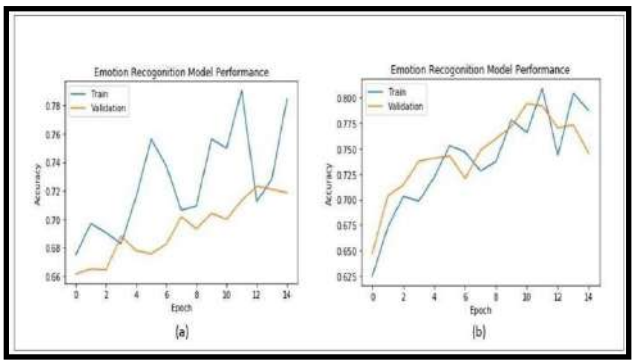

Fig. 4. Training and validation performance on 15 epochs

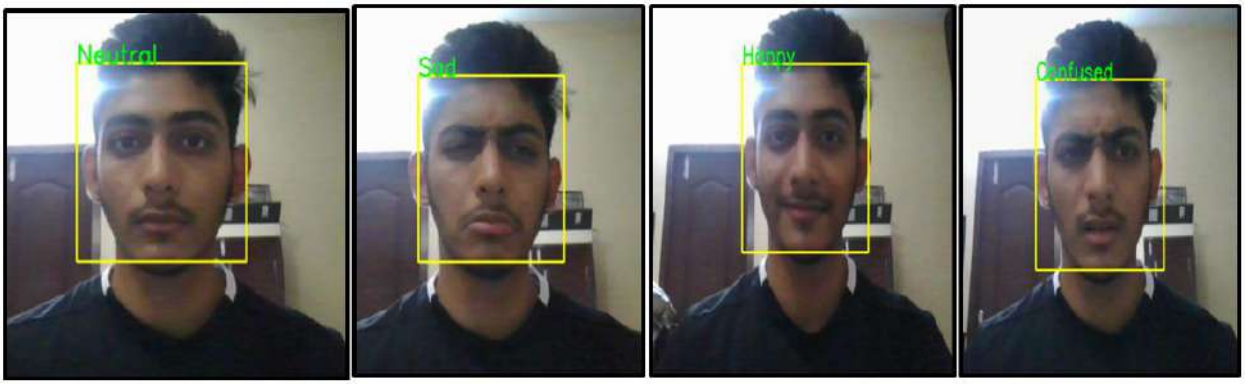

Fig. 5. The tested model output for emotion detection

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RESEARCH ARTICLE

Role of Agricultural Cooperatives in Sustainable Rural Development – A Survey of Recent Literature

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ABSTRACT

In the growing notion of rural development, agricultural cooperatives (ACs) have emerged as a need based financial intermediary to empower the economic conditions of farmers. This mechanism also helps to increase per capita income, reduce poverty and unemployment, and provide social welfare at the rural level. Any farmer can become a member of a cooperative bank by purchasing a certain number of shares within a specified period of time. Later, every member will be eligible to get economic benefits, which will be shared equally among the members. The study used 23 articles on the specific literature on ACs conducted at international, national, and state levels within and outside India from 2011 to 2021. Studies have pointed out that there is a positive impact of ACs on sustainable rural development. The present paper analysed only the research articles published in reputed journals, and an unpublished source was not covered.

Keywords: Agricultural Cooperatives, Rural Development

INTRODUCTION

Agricultural Cooperatives were brought into existence to uplift the living and operational conditions of farmers. In addition to this the agricultural cooperatives (ACs) are also working in the directions to improve the financial literacy, trade, commerce, marketing, accessing information, supply chain, agriculture loan extension (credit), training and social status, housing and political participation etc. In general the AGs are the voluntary association of farmers formed to meet the socio-economic, agriculture production and education need of the farmers. The goals of ACs are reduce poverty, supply of fertilisers, create employment, credit creation, assist vulnerable groups, increase income, minimise migration, provide livelihood, etc. This mutual as well as voluntary association has shown healthy potentials across India and rest of the world. The first cooperative was formed in 1904 by British government in kanaginahala, Gadag district of Karnataka state. Now ministry of Cooperative is looking after the ACs in their

respective states. In India around 41.49 percentage of people working with agriculture allied sectors and contributing 19.9 percent to GDP. To improve the primary sector the role of ACs is pivotal.

ANALYSIS OF THE STUDIES

The study focused on the specific literature on ACs conducted at international, national and state level within and outside India from 2011 to 2021. The present study covered 23 research papers and 99% of the studies concluded that ACs has positive impact on the sustainable rural development, helps farmers to achieve economic independency. Recent 23 literature survey on agricultural cooperatives is presented in a tabular form for easy reference.

Researcher/ Researchers	Title of the Study	Objective of the study	Conclusion of the study
Mansooreh Feisali and Mehrdad Niknami (2021)	Towards sustainable rural employment in agricultural cooperatives: Evidence from Iran's desert area	To examine the result of various function of ACs on sustainable rural employment.	ACs working towards to uplift the production, educational, social and living conditions of farmers epically at rural level. More over all this progress have positively correlated with sustainable rural development. The ACs also focus on entrepreneurial skills, training on animal husbandry, starts up program, self help groups etc.
Juha Junttila et al., (2021)	Keep the faith in banking: New evidence for the effects of negative interest rates based on the case of Finnish cooperative banks	To analyses the profitability of Finnish cooperative banks during the period of negative nominal interest rates from 2009-2014.	The increasing wholesale funding ratio is an important risk adjusted factor to measure the profitability of banks. The unconventional fiscal policies are curbing the profitability of the banks. Year by year the negative profit of the banking firms have becoming increasing. The risk adjustment ration of the banks is also negative and it is prolonged for many periods.
Xiaoyan Qian (2021)	Production planning and equity investment decisions in agriculture with closed membership cooperatives	The study has been undertaken to examine the Production planning and equity investment decisions in agriculture with closed membership cooperatives according to the farmers.	Cash constrained farmers require certain amount of money to purchase raw commodity to undertake agricultural activities. Relative equity requirement plays a significant role. The farmer's participation in decision is very useful for equity investment. The cooperatives should maintain liquidity to increase member's profitability. Cooperatives should maintain optimal capacity and equity investment policy rather than heuristic policies. There is a necessity to guides farmers' decisions on capacity and equity investment when transacting with closed co-ops.
Adalgiso Amendola et al., (2021)	Market Structure and Financial Stability: the Interaction between Profit-Oriented and Mutual Cooperative Banks in Italy	The study aim's on to relation between market structure and financial stability both theoretically and empirically between profit-oriented banks And mutual cooperative banks in the context of Italy.	Under the condition that mutual cooperative banks are not dominated by borrowers, there is an inverted U-shaped relation in which a less concentrated market structure increases Stability for both types of banks but a more concentrated market structure reduces it. The study on ACs will helpful to enhance the possible role cooperative members to achieve financial stability in cooperative banking system.
Andres Felipe Camargo	Rediscovering the Cooperative	The present study is undertaken to analyse	The cooperative movement has brought significant changes in the economic, business and social status of

Benavides and Michel Ehrenhard (2021)	Enterprise: A Systematic Review of Current Topics and Avenues for Future Research	the recent literatures in the field of cooperative enterprises.	cooperative members. The development will be inconsistent without steady and reliable source of income. The main challenge for the AGs is sustainable development due to transformation of technology, changes in the attitude of work force profile, corporate governance, changes in the organisation structure etc.
Erik Hanson and Michael Boland (2020)	Safety climate at agricultural cooperatives	To identifies the determinants of safety climate at agricultural cooperatives.	The occupational health and safety are main priority of agricultural cooperatives. Programs on discipline, inspection, safety, off the job etc are very helpful for the office bearers of agricultural cooperatives. Analysis on financial investment of agricultural cooperatives is the best practice.
Julius Manda et al., (2020)	Does cooperative membership increase and accelerate agricultural Technology adoption? Empirical evidence from Zambia	To identify the factors determine the decision of farmers to become cooperative member and adoption of technology in agriculture.	There is a positive relationship between adoption of technology and membership for agriculture cooperatives. The live stocks maintenance, education, availability of credit, off farmer employment, adoption of technology in agriculture etc. are double the income of agricultural cooperatives' members. In addition the cooperative also help to marketing, credit, input information among small farmers. Timely information on weather, rainfall etc is useful for farmers.
Wasiaturrahma et al., (2020)	Breadth and depth outreach of Islamic cooperatives: do size, Non-performing finance and grant matter?	The objective is to calculate the Breadth and depth outreach of Islamic cooperatives in east java.	The Tobit regression model was used to analyse the results. The study reviles that size, NPA, grants, financial leverage, number of branches, has greater impact on the cooperatives operation. Increase in the grants will make a positive impact the performance of Islamic cooperatives. There is also necessity of Islamic outreach cooperatives in the study area.
Jos Bijman and Gea Wijers (2019)	Exploring the inclusiveness of producer cooperatives	Impact of membership of producer agricultural cooperatives	The comprehensiveness of producer agricultural (PCs) is remaining as tough issues for the developing economies. Most of the poor farmers are excluded from primary producer agricultural (PCs) at Netherland. The cooperatives should be selective in choosing the farmers for membership. The cooperatives required to be more producer oriented rather than market oriented.
ZHANG Yan-yuan et., Al. (2019)	Farmers using insurance and cooperatives to manage agricultural risks: A case study of the swine industry in China	To study the factors affecting on farmers to buy agricultural insurance after joined to agricultural cooperates.	Farmers are exhibiting the risk adverse attitude towards agricultural insurance. And meanwhile they helping the govt. by paying insurance premium. The insurance company and govt. should understand the importance of ACs in the sustainable rural development. If possible they should introduce innovative crop insurance policies through ACs for the benefit of farmers.
Ephraim Clark (2018)	Cooperative banks: What do we know about competition	To measure the relationship between competition and	Cooperative banks are the prerequisites for the sustainable rural economic development. These financial institutions deserve special models for

	and risk preferences?	financial stability in European cooperative banking Between 2006 and 2014.	further development. There is s hump shaped relationship between loans and market power of the cooperative banks. Diversification is also equally important for the stability of banks.
Nikolaeva E (2018)	Efficiency Analysis of Agricultural Cooperation in Russia	To identify the reasons for the efficiency of cooperative form of agricultural cooperatives in Russia.	From 2014 the efficiency of cooperatives and non cooperatives efficiency and performance of cooperatives has decreased in Russia. The cooperative fails to attract the small businessmen's, farmers in rural Russia. Government need to revise the present policies and develop suitable instruments and institutions to support the cooperatives.
Tarcisio Pedro Da Silva (2017)	Financial and economic performance of major Brazilian credit cooperatives	The study aimed to analyze which the financial and economic performance of Brazil's largest credit unions by using CAMEL model.	There is a positive relationship between the use of the CAMEL model variables and the measurement of the financial and economic performance of the credit cooperatives. Thus, the expected result would consider two aspects :the first would show that the higher the rates of the economic and financial performance of CAMEL model, the higher the performance addressed to the capacity for growth in loans activity to the associate clients; the second would indicate that the greater the gap in the capital growth indicator the greater the loan capacity dispensed to associate clients
Abdul Rehman (2017)	Is credit the devil in the agriculture? The role of credit in Pakistan's agricultural sector	The aim of this study was to use an econometric analysis to investigate the relationship between the agricultural gross domestic product (AGDP) and variables, loan distributed by ACs in Pakistan	Pakistan's economy is depending on primary sector epically on agriculture. Agriculture sector contribute 60% to the countries annual GDP. But in the study area the contribution from Agriculture sector is depleting due to failure of up gradation of technology, loans distributed through AGS are not recovering on time because there is negative correlation between the cropped area and the AGs loan distributed area. So there was mutualisation of loan from the borrowers. The govt of Pakistan need to form the new schemes or mechanism for distribution and control of credit through the agricultural Cooperatives.
Paata Koguashvili (2016)	Support for agricultural cooperatives is an urgent necessity	Effectiveness of cooperatives for agricultural management, planning and control.	Most cooperatives are in start up stage with scarcity of financial resources. Non availability of timely credit from commercial banks is the main barrier for agricultural cooperatives. Unless the effective measures the agricultural cooperatives would be adversely effected.
Julie A. Hogeland (2015)	Managing uncertainty and expectations: The strategic response of U.S. agricultural cooperatives to	To foster cooperative and farmer expectations of industrialization and cooperatives	The cooperatives are like family i, e members are the office bearers and working for mutual cooperation. Cooperatives should ensure high cooperative life, high cohesiveness between members. The ideology of cooperative union's directors is spoiling the concept of cooperatives. Cross country cooperative's

	agricultural industrialization		comparative evaluation helps cooperative development.
Bouker Maroua (2015)	The Impact Of The Multi-Stakeholders Governance On The Performance Of Cooperative Banks: Evidence Of European Cooperative Banks	The present study has undertaken to analyse the past and present indicators of cooperative systems in Europe.	Cooperatives banks showing interest in development of local people hence they become poor man's bank. There is a positive relationship between membership and profitability of cooperatives. To strengthen the banking system in Europe the requirement of cooperative banks along with commercial banks is necessary. The cooperatives have limited area to operation. And the cooperative banks performance should not compare with commercial bank.
Liliana-Aurora Constantinescu (2015)	Cooperative Spirit in the XXI Century European Cooperative Culture	The understand the cooperative spirit in Europe from 1960 to 2013	The cooperative movement has the following culture in Europe; Justice, Equity, equality, union, democracy, social emancipation, human dignity and care, association of people, unity and identity. The cooperatives adopt the principal of unity in diversity and socio-economic growth of members.
Davide Salvatore Mare and Dieter Gramlich	Risk exposures of European cooperative banks: a comparative analysis	To focus the risk coverage of CBs in Germany, Austria and Italy.	CBs are exposed for financial risk very frequently. The integrated literature review on CBs in the selected area has analyzed the joint behaviour of the bank employees and overall system. The main reasons for financial risk of AGs are; weak liquidity position, declining in the profit, no risk management, no risk prediction techniques.
Debdatta Pal and Arnab Kumar Laha (2014)	Credit off-take from formal financial institutions in rural India: quantile regression results	To Assessing Policy Interventions in Agri-Business and Allied Sector Credit versus Credit Plus Approach for Livelihood Promotion.	Superior loan size were absolutely and notably connected with higher operation costs, which means that borrowers may need to deserve significant operating expense in common visits to bank branches to get credit facilities. There is significant disparity in the quantum of loans received from official creditors. Despite government pushes for financial inclusion, the sharing of credit from the proper monetary sector leftovers skewed towards resource-rich rural households.
Jurat Ismail, Wei Xianhua (2013)	Investigation and Analysis on Current Situation of Rural Cooperative Finance in Xinjiang	To examine the current situation and basic problems of cooperative finance in Xinjiang	There is poor ecological environment of Rural cooperative finance in Xinjiang, some differences in development between southern and northern Xinjiang and lack of Rural cooperative financial institutions at basic level. The study suggest that the Rural cooperative support agricultural labours, increase the credit hours, group lending, need of special financial assistance for cooperative.
Meizhang xiangyuGuo (2010)	Study on Functions of the Agriculture Cooperative in Food Safety	The goal of this paper was to investigate the environment pressure on food crops and identify the role of AGs to achieve food security.	AGs are the effective actors to achieve the food safety and security. The cooperatives are helping the govt. ensure the food safety through inspection and control. AGs required increasing training on market information, food processing to farmers; this will bring awareness among farmers to become conscious food grains producers. Cooperatives should follow

			the principle of 'quality first credit best'.
Yadollah Rajaei et al., (2011)	Assessing effective factors in development of entrepreneurship in agricultural cooperatives of Zanjan province	To identify the entrepreneurial score of agricultural cooperatives and identify the factors affecting to strengthen the agricultural cooperatives.	According to the study the strength of agricultural entrepreneurship is affected by compliance of entrepreneurship, employment laws, and government agencies. The AGs are helping the agricultural entrepreneurs through providing the financial support as well as tax benefits etc. It is recommended that the AGs members and managers should provide specialized training on agricultural entrepreneurship.

Above studies have point out there is positive impact of ACs on sustainable rural development. Many studies observed that the main reasons for financial risk of AGs are; weak liquidity position, declining in the profit, no risk management, no risk prediction techniques etc. the same scenario is also applicable to India.

CONCLUSION

Most of ACs members are scarcely literate, to make them financial sound, achieve financial literacy, the ACs role is instrumental. There is a need of strong financial intermediary at grass root level in developing country like India. Major percent of Indian population is depending on agriculture and it has contributed 20.19 % to Indian GDP in the year 2021-22. Hence cooperative banks are the prerequisites for the sustainable rural economic development. To make agriculture and allied activities more profitable the ACs should be strengthen across India.

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RESEARCH ARTICLE

An Empirical Study on Health Infrastructure of India to Fight against COVID-19

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ABSTRACT

The pandemic Coronavirus 2019 has globally affects in around 202 countries worldwide. In India, the COVID-19 infected people are 2113 as on 3rd April, 2020 . The health infrastructure is vital for containment and mitigation of CONVID-19. The health infrastructure of India against COVID-19 is an essential factor to determine the quality of services available to patients. The present paper is an empirical approach to measure the health infrastructure available in the different states of India. In this paper a composite index is developed which used to measure the health infrastructure in terms of fight against COVID-19. To attain the objective mentioned above, health infrastructure index is developed with fitted exponential distribution. This index highlighted the condition of health infrastructure in the different states of India. On the basis of the distribution of the index the states are classified into three groups i.e. poor infrastructure, moderate infrastructure and high infrastructure in terms of health. This study provides the quantification of state level scenario on health infrastructure to prevent the COVOD-19 before the government to promote occupational health related schemes at workplace with facility.

Keywords: Health Infrastructure, Classification, Probability Distribution, Health Infrastructure Index.

INTRODUCTION

The World Health Organization (WHO) reported that 750890 confirmed cases of Coronavirus 2019 (COVID-19) and deaths 36504 as on 31st March, 2020 [1]. Initially the pandemic COVID-19 found in China. Now , the pandemic COVID-19 affected around 203 countries/territories/ areas. WHO provided the transmission of COVID-19 is due to high mobility through travel, person to person transmission. However an advisory on exit and entry screening of tourist or visitors for the signs and symptoms of respiratory contagion with temperature screening to identify the suspects for laboratory test for confirmation of COVID-19.

Objective of the study

The main objectives of the study are

(I) To quantify the level of Health infrastructure in the different states of India.

(II) To develop a weighted index of health infrastructure to categorize the states as per their infrastructure.

DATA AND METHODOLOGY

Data

The information about relevant data for the study is collected from the 14th issue of National Health Profile 2019 from website of ministry of Health and Family Welfare (mohfw.nic.in). The report provides a wide range of up-to-date factual data on diverse aspects of infrastructure to prevent COVID-19. Efforts have been made to present the latest available data covering up to 2019.

METHODOLOGY

Health Indicators

The following health infrastructure indicators are used for the study

(i) Hospitals per 10000 population,

(ii) Beds per 10000 population,

(iii) Dispensaries per 10000 population,

(iv) Registered practitioners per 10000 population

The Health composite infrastructure index is defined as,

$$HII_i = \sum w_i x_{ij} \quad (1)$$

where, HII_i is the composite infrastructure index of medicine for the i^{th} observation, x_{ij} is the percentage of the i^{th} aspect of infrastructure for the j^{th} state and it is defined by

$$X_{ij} = \frac{y_{ij}}{y_i} * 100 \quad (2)$$

Where , y_{ij} = value of the i^{th} indicator for the j^{th} state of India

y_i = value of the i^{th} indicator for India.

W_i represents the weight associated with the i^{th} basic facility and is given by the following

$$W_i = \frac{\sigma_i^{-2}}{1+\sum \sigma_i^{-2}} \quad (3)$$

The choice of the weights in this manner would ensure that large variation in any one of the indicators would not unduly dominate the contribution of the rest of the indicators and distort the inter district comparisons.

Distribution of the Weighted Index of Health Infrastructure of Alternative system of medicine

Iyengar and Sudarshan (1982) assumed that the development index followed the Beta distribution [2]. Vidwan (1983) empirically showed a better classification under a normal distribution[3]. Hence, the assumed distribution played a crucial role in obtaining the empirical outcomes [4]. For testing the hypothetical distribution of the weighted index of infrastructure of alternative system of medicine (HII_i), one may use the chi-square test of goodness of fit. As $HII_i \in [0, 1]$, the values of the indices are essentially continuous in nature. To model the empirical frequency, the range [0, 1]

can be divided into non-overlapping class intervals, and the chi-square test of goodness of fit can be conducted after obtaining the frequency within each class interval. The observed frequency can be compared with the theoretical frequencies expected under the hypothetical distribution. Although the interval setting could be arbitrary and converting the scale from continuous to discrete might have reduced the precision, the approach outlined above has been commonly used in practice [5]. The Kolmogorov Smirnov (K-S) test statistic, could also be applied in this case as the indices are continuous in nature. Different authors have proved that the K-S statistic is more appropriate for continuous data compared to the chi-square test of goodness of fit[6]. The test statistic is given by,

$$D_n = \max | S_n(x) - F(x) | \quad \ldots(4)$$

where $S_n(x)$ and $F(x)$ are empirical and theoretical distribution functions respectively. However, for performing the K-S statistic the theoretical distribution needs to be completely specified i.e. the valued of the parameters needs to be known. In this exercise the parameters are estimated form data. The critical value of D_n for α level of significance depends on the number of observations and may be denoted by D_{α^n} . If the number of observations are over 35, as the case here, the critical value at 5 percent level of significance ($D_{0.01,n}$) is 1.63/√n. Thus, D_n value greater than 1.63/√n, will indicate that the fitted distribution is significantly different from the theoretical distribution. Thus, both the tests viz. the K-S test and chi-square test can be used to verify the appropriate distribution to which the HII_i values, fit. The interval [$F(x)- D_{\alpha^n}, F(x)+ D_{\alpha^n}$] provides the 100(1-α)% confidence band for $F(x)$ which can be used as a visual tool for goodness of fit[7]. After deciding about the probability distribution of HII_i it is important to find two real numbers $c, d \in [0, 1]$ to divide three linear intervals namely [*0, c*], [*c, d*] and [*d,* 1] with the same probability weight of 33.33%, i.e.,

$P[0 \le HII_i \le c] = 0.3333$ …(5)

and, $P[0 \le HII_i \le d] = 0.6666$ … (6)

Thus, $P[c \le HII_i \le d] = 0.3333$ using (5) and (6)

These intervals has been used in this study to characterize the various stages of deprivation as follows:

(i) Low Deprivation if $0 \le HII_i \le c$;

(ii) Moderate Deprivation if $c \le HII_i \le d$

(iii) High Deprivation if $d \le HII_i \le 1$

ANALYSIS AND RESULT

Since the values of HII_i lies between 0 and infinity, one may select the one parameter exponential distribution as a probable distribution. The exponential distribution probability density function is given by,

$$f(x) = \lambda e^{-\lambda x}, \quad x > 0 \text{ and } \lambda > 0 \quad (7)$$

= 0, otherwise

Based on the values of *HII* for all states, the estimated values of *a* and *b* are obtained using the method of maximum likelihood (Johnson and Kotz, 1970). The estimated values are given by,

$$\hat{\lambda} = \frac{1}{m_1} \quad (8)$$

Where, m_1 = mean of all *HII*s

Based on the empirical data for this investigation, the estimated model parameters arem $\hat{\lambda} = 2.9429$. The K-S test is also used to test if the *HII* values fit to the exponential distribution specified by the parameters already estimated from the data. The value of the statistic,

$$D_n = \max | S_n(x) - F(x) | = 0.259$$

which is insignificant at 5 percent level.

To reduce potential subjectivity in the model selection, the empirical distribution function plot is employed to triangulate the findings from the chi-square test and to visualize the results of the K- S test. The closeness of the step function (EDF) to the CDF curve and the step function lying within the bounds reconfirmed the model fitness to the empirical database.

Footnote

The graph is created with R, an open source environment and language for statistical computing and graphics <http://cran.r-project.org/>. On the basis of the above categorization, the different states of India can be revealed in the following table:

CONCLUSION

In order to quantify the states wise infrastructure indicators mentioned above. All these indicators are aggregated using a composite index. The distributional pattern of the of the infrastructure index is recognized to ease classification of the state based on the infrastructure. The different indicators that are considered can be weighted based on their relative importance. The outcome of the study reflects that the low infrastructure of different facilities mentioned above in some of the states crop up mainly because of the unequal distribution of facilities. The infrastructure index is considered necessary to help Ministry of health & Family welfare (MOHFW) to formulation and implementation of various development policies by the government of India to prevent the COVID-19. Continuous follow-up the patients, it is essential that the health infrastructure should be improve to prevent the COVID-19.

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Appendix-I Values of the Indicators and Health Infrastructure Index

Name of State / UT	Doctors	Hospital	Hospital Bed	Health Infrastructure Index
Andhra Pradesh	0.957842	0.048323	4.333702104	0.066527341
Arunachal Pradesh	3.544735	1.407563	15.52193474	1.314339592
Assam	1.733711	0.349479	4.886429844	0.342811571
Bihar	0.228371	0.093819	0.954055949	0.08745266
Chhattisgarh	0.560887	0.073819	3.246660402	0.076169228
Goa	4.116728	0.274875	19.25401557	0.322780685
Delhi	4.930752	0.058925	13.1812882	0.147347056
Gujarat	0.844883	0.067591	3.112871642	0.076138696
Haryana	0.941952	0.240345	4.044132714	0.230926771
Himachal Pradesh	2.054436	1.084775	16.79166178	0.999852031
Karnataka	0.755003	0.425231	10.43193564	0.390970037
Kerala	1.477362	0.360951	10.71686928	0.348017715
Madhya Pradesh	0.54717	0.055456	3.709734293	0.059650622
Maharashtra	1.044525	0.106383	7.697556848	0.114329732
Manipur	3.604625	0.098397	4.680436399	0.156685102
Mizoram	3.575713	0.736417	16.34027038	0.720871094
Odisha	0.937615	0.393798	4.038068482	0.366672368
Puducherry	5.021427	0.100429	25.60210498	0.185834796
Punjab	1.114961	0.228281	6.00258118	0.223587933
Rajasthan	0.908094	0.358111	5.912476029	0.334513757
Tamil Nadu	0.93719	0.157688	10.04592787	0.157670923
Telengana	1.059378	0.221742	5.391446565	0.21672727
Chandigarh	12.58666	0.078776	32.87587781	0.312718409
Jammu and Kashmir & Ladakh	2.951651	0.104013	5.303225424	0.149050192
Uttar Pradesh	0.460796	0.198604	3.267652924	0.184690907
Jharkhand	0.472664	0.146307	2.842839932	0.138628994
Meghalaya	1.761928	0.472859	13.42378501	0.452567025
Nagaland	1.496416	0.162262	8.473682455	0.172515339
Sikkim	3.937002	0.48478	22.91687784	0.505109184
Tripura	3.054309	0.379357	10.77033031	0.394752909
Uttarakhand	1.206402	0.412905	7.640545373	0.388774036
West Bengal	0.894872	0.158723	7.963135122	0.15777021
A & N Island	1.750641	0.729434	2.601646575	0.679456322
D & N Havelli	2.551698	0.312453	16.11735727	0.325829583
Daman & Diu	2.643784	0.224049	10.75437457	0.249356937
Lakshadweep	4.018179	1.247021	41.56736685	1.181375394

Table 1 State Classification Range

Infrastructure Category	Range of Infrastructure Index
Low Infrastructure	Less than 0.1377433
Moderate Infrastructure	Between 0.1377433 to 0.3736096
High Infrastructure	Greater than 0.3736096

Table 2 State classification

Type of Category	States
Low Infrastructure	Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Madhya Pradesh, Maharashtra
Moderate Infrastructure	Assam, Goa, Delhi, Haryana, Kerala, Manipur, Odisha, Puducherry, Punjab, Rajasthan, Tamil Nadu, Telengana, Chandigarh, Jammu and Kashmir & Ladakh, Uttar Pradesh, Jharkhand, Nagaland, West Bengal, D & N Havelli, Daman & Diu
High Infrastructure	Arunachal Pradesh, Himachal Pradesh, Karnataka, Mizoram, Meghalaya, Sikkim, Tripura, Uttarakhand, A & N Island, Lakshadweep

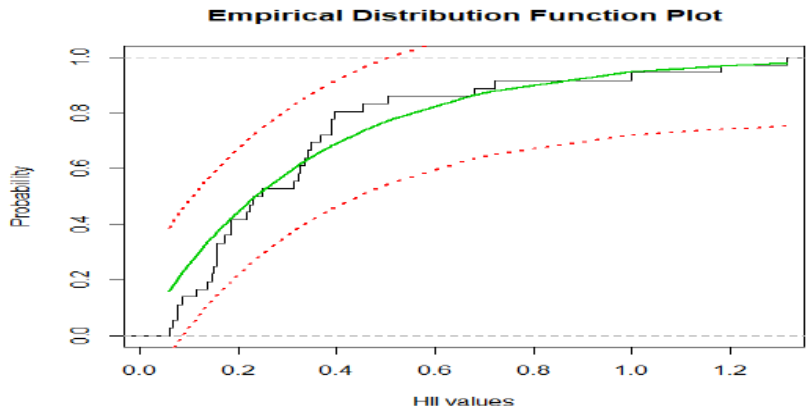

Figure 1. Visualizing the goodness of fit of HII values to distribution using empirical distribution function plot

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RESEARCH ARTICLE

Within-Breed Genetic Diversity and Bottleneck Analysis of Umblachery Breed of Cattle by Automated Fragment Analysis of Microsatellite Markers

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ABSTRACT

Umblachery breed of cattle is a draught breed of Tamil Nadu in south India developed mainly for its short stature to work in marshy fields. Thirty animals were randomly selected from the field and 10 FAO recommended microsatellite loci from different chromosomes were used for within breed diversity analysis. This study used forward primers with fluorescent labels (FAM, TET, TAMRA and HEX) at their 5 prime end and the amplicons were resolved by Automated fragment analysis method using ABI 3730XL Genetic Analyzer. A total of 76 different alleles were observed across 10 loci ranging from 4 alleles (ILSTS011) to 11 alleles (TGLA122) with a mean of 7.6 ± 0.71. The effective number of alleles ranged from 3.0347 (ILSTS011) to 9.7474 (TGLA122) with a mean of 6.2395 ± 0.86. The mean polymorphism information content (PIC) was 0.67 ± 0.06 indicating the markers chosen are highly informative. High genetic variation within the population was observed with an overall mean expected heterozygosity of 0.7124 ± 0.06.The population was also found to be outbred (F_{IS} value of -0.0194 ± 0.07) and non-bottlenecked as the mode-shift analysis produced an L-shaped curve.

Keywords: Draught Breed, Fluorescently Labeled Microsatellite Markers, Genetic Variation, Heterozygosity, PIC, Bottle-Neck Analysis.

INTRODUCTION

Umblachery is a medium-sized draught breed of cattle found mainly in Thiruvarur, Thanjavur and Nagapattinam Districts of Tamil Nadu in south India. This breed was developed for the need of short- statured draught animals to work in marshy paddy fields. The bullocks of this breed are capable of working for 6 to 7 hours continuously under hot sun. The coat colour is red at birth which progresses to grey shades at 3 to 4 months of age[14]. The white markings on face, limbs and tail are characteristics of the breed. The lower limbs have white markings which appear as socks. As per the breed data sheet of Domestic Animal Diversity Information System (DAD-IS) of FAO (2022)[1], the population was estimated to be between 31195 to 42390 and at present reported as 'not at risk'. The gene diversity study on Umblachery was already carried out by Karthickeyan [5] and Thiagarajan [18]. Yet, it is essential to keep regular check on the genetic diversity of this breed to avoid loosing of this valuable native germplasm which is under regular threat of crossbreeding and mechanization of agriculture and thereby taking necessary conservation measures. Moreover, this study has used advanced technique where fluorescently-labeled primers were used and the genotypes were accurately measured using an automated DNA analyzer, in order to efficiently score different alleles in various microsatellite regions in the genome of the population.

MATERIALS AND METHODS

Genomic DNA isolation

In this study 10 microsatellite markers recommended by FAO [4] were used to characterize Umblachery breed of cattle. A total of 30 animals were randomly sampled from the breeding tract. Genomic DNA was isolated from the blood samples of those animals using modified phenol-chloroform method [15]where DNAzol was used instead of proteinase-k. The isolated DNA was quality checked and quantified by Nanodrop spectrophotometer (Thermo Scientific, USA).

Multiplex PCR and Fragment analysis

The forward primers used in the study were fluorescently labeled using FAM-BLUE, HEX-green, TET-green and TAMRA-black dyes at 5' end to perform multiplex PCR. All the 10 primers were grouped into two panels based on the size of the product, fluorescent dye and annealing temperature. The PCR reaction mix (25µl) consisted of red dye master mix (AMPLIQON) of 2X concentration, panel of 5 primer sets (concentration of each varying from 4 to 7 picomoles), template DNA(50ng) and nuclease free water. The PCR conditions had initial denaturation at 95°C for 5 minutes, then 35 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds and extension at 72°C for 40 seconds and then with a final extension of 72°C for 5 minutes. All the amplicons were genotyped by fragment analysis method, where the PCR products were diluted in the ratio of 1:60 and mixed with Hi-diformamide and GS500liz size standard. Then the products were separated by capillary electrophoresis (CE) and were resolved by comparing with the size standard. The DNA sizing and allele calling were done using Gene Mapper® software as well as Peak Scanner 2 software.

Statistical Analysis

All the genotypes were analyzed for allelic diversity parameters *viz*., observed number of alleles (na), effective number of alleles (ne), allele frequency, most frequent alleles (MFA), observed heterozygosity value (Ho), expected heterozygosity value (He) and heterozygote deficit within the breed (FIS), gene flow (Nm), Hardy-Weinberg equilibrium (HW) using MS Analyzer [2] and popgene - version 1.31 [19]. The input data for both the programs were created with the help of GENALEX 6.2 software[11]. Polymorphism information content (PIC) of the markers was estimated with the help of PIC calculator[10]. Bottleneck analysis was also performed to find any recent reduction in the population [12]. BOTTLENECK program assumed three mutation models *viz.,* the Infinite Allele Model (IAM), Two Phase Model of mutation (TPM) and Stepwise Mutation Model (SMM) to test if all the markers are under Hardy-Weinberg Equilibrium and genotypic linkage equilibrium. The models were also assessed by "sign test," "standardized differences test" and "Wilcox on sign-rank test" to predict hetrozygosity excess with null hypothesis

of mutation drift equilibrium of the population. Mode shift indicator test was also performed, which depended on distribution of allele frequency to check recently bottlenecked event in the population [1; 8].

RESULTS AND DISCUSSION

Fragment analysis

The ten microsatellite markers chosen in the study were amplified in 2 multiplex panels in 30 animals which resulted in a total of 300 PCR reactions. The electrophoregram files retrieved after fragment analysis were scored manually for each marker for their allelic sizes based on their fluorescent labels and height of the peak in their respective size range. Each marker was observed to follow a typical pattern of peak(s) in all the samples and representative electrophoregrams of microsatellite locus (ILSTS006) is shown in Figure 1.

Number of alleles

Umblachery breed of cattle assessed across 10 different microsatellite loci had revealed a total of 76 different alleles (No) across 10 loci ranging from 4 alleles in ILSTS011 to 11 alleles in TGLA122 with a mean of 7.6 ± 0.71 (Table 1). The mean number of alleles was similar to that of an earlier reports on Umblachery (7.63) by Manomohan [9] and on other two south Indian cattle breeds, Hallikar (7.55±0.65) and Krishna valley (7.35± 0.50) as stated by Hepsibha [4] and Karthickeyan [6] respectively. The mean number of alleles were comparatively higher (8.784± 0.25) in an another study where 11 different Indian cattle breeds [17] of India were investigated. The effective no. of alleles by Stepwise Mutation Model in Umblacherry breed of cattle was ranging from 3.035 (ILSTS011) to 9.75 (TGLA122) with a mean of 6.24 ± 0.86 (Table 1). The overall mean was comparatively higher than Hallikar (3.67± 0.31) [4] and Krishna valley (3.9± 0.29) [6] breeds of cattle. These results show that this breed has not lost its allelic diversity. The results also indicate that more than 80 per cent of the alleles are occurring at high frequency and thus retained in the population.

Heterozygosity

Gene diversity is an appropriate measure of genetic variation within the population and expressed as expected heterozygosity. The observed heterozygosity in Umblacherry ranged from 0.400 (HAUT024) to 0.933 (HEL 9) with a mean of 0.713 ± 0.06. The expected heterozygosity values ranged from 0.431 (ILSTS011) to 0.875 (TGLA122) with a mean of 0.712 ± 0.07 (Table 1 and Figure 3), which was exactly similar to the report on the same breed (0.710) by Manomohan [9] indicating a high level of genetic variation still existing in the breed.

Polymorphism information content (PIC)

The breed has an overall mean polymorphism information content of 0.670 ± 0.06 (Table 1 and Figure 3) with a range of 0.359(ILSTS011) to 0.846 (TGLA122). Eighty percent of the markers had PIC values above 0.5, which suggests that the markers chosen for the study are highly informative. Hepsibha [4] and Karthickeyan [6] also had shown similar PIC values in Hallikar and Krishna valley breeds of cattle in south India with mean PIC values of 0.6367 ± 0.03 and 0.6640 ± 0.03 respectively

Inbreeding co-efficient(F_{IS})

Out of ten markers investigated, four markers had suffered heterozygosity deficit and the remaining six had negative F_{IS} values with an overall mean of -0.0194 ± 0.07 (Table 1 and Figure 4) across all 10 loci, which is a good indicator of population being outbred. Hence, necessary conservation measures are required to save the superior genes possessed in our native germplasm. According to Sharma *et al*., 2015, heterozygote deficit was highest in Ongole (22.1 per cent) and lowest in Ponwar (1.4 per cent) and the global mean of F_{IS} for four Algerian breeds was 1.4 percent [13].

Hardy-Weinberg equilibrium

Out of 10 Loci, 8 were under Hardy-Weinberg equilibrium (HWE) and only 2 markers HAUT024 and ILSTS033 had deviated as indicated from the highly significant chi-square (χ^2) values; while more number of loci had deviated in Hallikar (7 out of 22 loci) and Krishna Valley (14 out of 23 loci), the other south Indian cattle breeds as reported by Hepsibha [4] and Karthickeyan [6]. The results infer that this breed has neither been much influenced by any other systematic forces like selection, mutation and migration nor suffered from small population size.

Bottleneck analysis

Bottleneck analysis of Umblachery cattle with microsatellite markers revealed the expected numbers of loci with heterozygosity excess as 5.95, 5.98 and 5.93 in IAM, TPM and SMM, respectively under sign test. The null hypothesis for mutation drift equilibrium under sign test, Wilcoxon test were accepted for all the three models. While, under standardized differences test, the T2 values for SMM rejected the null hypothesis as the probability value was less than 0.01 (Table 2). The mode-shift analysis found the population to be non-bottlenecked as the allele frequency distribution formed a L-shaped curve (Figure 5) due to abundance of low frequency alleles as stated by Luikart [8]. This indicates that the breed has not undergone any recent drift in the population. The findings were similar to Red steppe [7] and Nimari [16] cattle which also had not undergone recent reduction in the population.

CONCLUSIONS

Current study in Umblacherry had revealed the existence of high level of genetic variation with maximum number of alleles having higher frequency. The bottleneck analysis and the heterozygosity level in the population align with the DAD-IS 2020 report, that the animal at present is 'not at risk'. Yet consistent efforts on genetic improvement of the breed through appropriate breeding programmes and strict conservative measures are necessary to protect the germplasm.

Authors' Contributions

Conceptualization KSMK; Field surveys & Sample collection KDM; Sample processing HP & KDM; Funding acquisition KSMK &HP; Fragment analysis, Scoring,& Statistics HP; Writing - original draft HP; Research advisory JHSV; Writing - review and editing KSMK. All authors read and approved the final manuscript.

Ethical Approval

This research involves collection of data and blood samples from Umblacherry breed of cattle from the field. Blood samples were collected through Madras Veterinary College (already authorized to treat and do research on animals) and by Dr. Kousalya Devi, a Veterinarian and research scholar in the same institute and also a co author of this paper. The remaining research involves non-infectious methods.

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Table 1. Genetic Diversity of Umblachery cattle assessed with microsatellite markers.

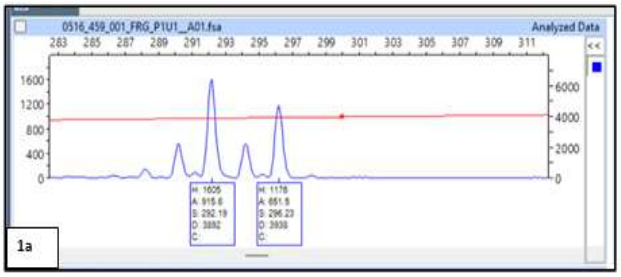
Microsatellite loci	5′ fluorescent labelling	Range of alleles (bp)	N_o	N_e	Most Frequent Allele (MFA)	Frequency of MFA	Ho	He	PIC	F_{IS}	HWE	
											χ^2	df
HAUT024	HEX	104 to 136	8	3.72	118	0.667	**0.400**	0.547	0.522	**0.264**	137.35**	28
ILSTS006	FAM	288 to 302	7	4.93	292	0.500	0.900	0.689	0.640	-0.320	21.70^{NS}	21
ILSTSO33	TAMRA	134 to 154	9	7.91	148	0.283	0.633	0.837	0.801	0.238	57.89**	36
INRA5	FAM	135 to 149	7	6.71	139	0.300	0.767	0.797	0.751	0.030	21.013^{NS}	21
TGLA122	TET	136 to 162	**11**	**9.75**	136	0.267	0.900	**0.875**	**0.846**	**-0.038**	66.44^{NS}	55
BM1824	HEX	179 to 193	5	3.30	181	0.700	0.500	0.480	0.437	-0.052	3.08^{NS}	10
HEL1	TAMRA	104 to 124	8	7.36	110	0.300	0.700	0.820	0.780	0.141	26.32^{NS}	28
HEL9	FAM	148 to 168	9	9.32	166	0.200	**0.933**	0.868	0.836	-0.085	27.58^{NS}	36
ILSTS011	HEX	260 to 268	**4**	**3.03**	262	**0.717**	0.533	**0.431**	**0.359**	-0.252	6.04^{NS}	6
MM8	HEX	120 to 144	8	6.36	136	0.333	0.867	0.781	0.735	-0.120	25.05^{NS}	28
Mean ± Std.Error			7.6 ± 0.71	6.24 ± 0.86			0.713 ± 0.07	0.712 ± 0.06	0.670 ± 0.06	-0.019 ± 0.07		

** $p \leq 0.01$ means population departed from HWE; ^{NS} $p > 0.05$ means population had not departed from HWE

Table 2. Test for null hypothesis under three mutation models for bottle neck analysis

Models	Sign test				Standardized differences test		Wilcoxon test		
	Hee	Hd	He	p	T2	p	P (one tail for Hd)	P (one tail for He)	P (two tails for He and Hd)
IAM	5.95	3	7	0.37	1.393	0.08	0.903	0.116	0.232
TPM	5.98	4	6	0.63	-0.331	0.37	0.652	0.384	0.770
SMM	5.93	5	5	0.38	-3.587	0.00	0.138	0.884	0.275

IAM-Infinite allele model; TPM-Two phase model; SMM- Stepwise mutation model; Hee – Expected Heterozygosity Excess, Hd- Heterozygosity deficiency, He- Heterozygosity Excess



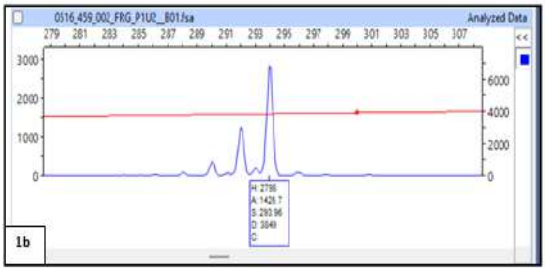


Figure 1a and 1b: Electrophoregrams of microsatellite marker ILSTS 006 (FAM-labeled) in two different animals. 1a- heterozygote alleles of two different sizes (two peaks); 1b- homozygote alleles of same size (single peak)

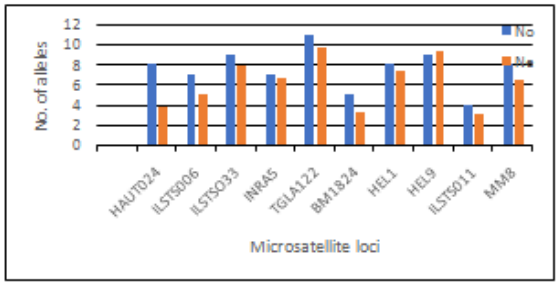


Figure 2: Observed and expected number of alleles in Umblachery breed of cattle

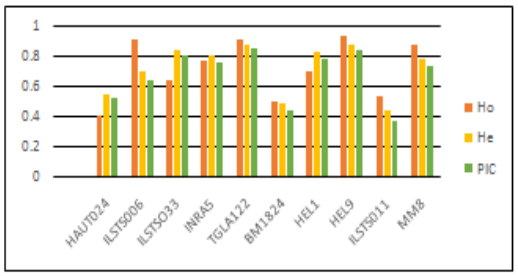


Figure 3: Observed (H_o), expected heterozygosity (H_e) and Polymorphism information content(PIC) values in Umblachery breed of cattle

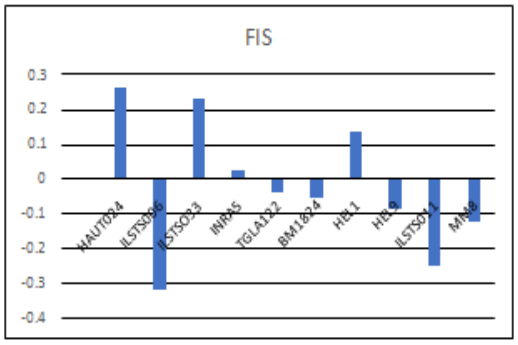


Figure 4: Fixation index (F_{IS}) as a measure of heterozygote deficiency or excess in Umblachery breed of cattle

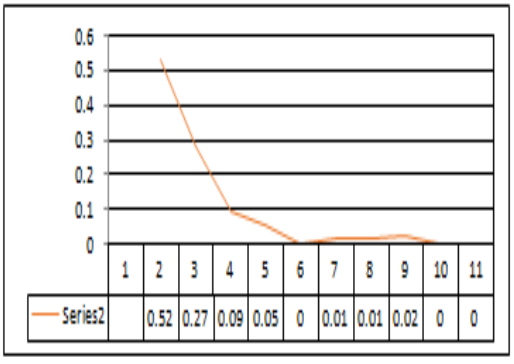


Figure 5. Graphical representation of allele frequency distribution in Umblachery cattle showing L-shaped curve.